EVALUATION OF TRACE ELEMENT STATUS IN HEMODIALYSIS PATIENTS

Dissertation Submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

in partial fulfillment of the regulations

for the award of the degree of

M.D. (BIOCHEMISTRY)
BRANCH – XIII

GOVT. STANLEY MEDICAL COLLEGE & HOSPITAL
CHENNAI, INDIA.

MARCH 2008

CERTIFICATE

This is to certify that the dissertation entitled **"EVALUATION OF TRACE ELEMENT STATUS IN HEMODIALYSIS PATIENTS"** is the bonafide original work of **Dr. G.MURUGAN** in partial fulfillment of the requirements for **M.D. (BIOCHEMISTRY) BRANCH – XIII Examination** of The Tamilnadu Dr. M.G.R. Medical University to be held in March 2008.

DR.MYTHILI BASKARAN, M.D.
DEAN
Govt. Stanley Medical College
and Hospital,
Chennai-600 001.

Dr. P. JAYANTHI, M.D.
PROFESSOR & HEAD
Department of Biochemistry
Govt. Stanley Medical College
Chennai-600 001.

DECLARATION

I, **Dr. G. MURUGAN,** solemnly declare that dissertation titled, "**EVALUATION OF TRACE ELEMENT STATUS IN HEMODIALYSIS PATIENTS"** is a bonafide work done by me at Govt. Stanley Medical College & Hospital during 2005-2008 under the supervision of **Dr. P. JAYANTHI, M.D.** Professor and Head, Department of Biochemistry and guidance of **Dr. RENGARAMANI M.D.,D.G.O.,** Professor of Biochemistry, Stanley Medical College, Chennai-600 001.

The dissertation is submitted to The Tamilnadu, Dr. M.G.R. Medical University, towards partial fulfillment of requirement for the award of **M.D. Degree (BRANCH – XIII) in Biochemistry.**

Place: Chennai.

Date:

(Dr. G.MURUGAN)

ACKNOWLEDGEMENT

There is hardly any task which is more pleasant than acknowledging my gratitude to all those who have helped me in so many ways in preparing this dissertation.

First of all I wish to express my most sincere appreciation and thanks to **Dr. MYTHILI BASKARAN, M.D.,** Dean, Government Stanley Medical College and Hospital, Chennai – 600 001 and **Dr. T. RAVEENDRAN, M.D.,** former Dean for permitting me to do this dissertation.

I am highly obliged and indebted to **Prof. Dr. P. JAYANTHI, M.D**., Professor and Head, Department of Biochemistry, Stanley Medical College for her immense support and valuable suggestion offered to carry out this procedure and permitting me to use all the needed resources for this dissertation work.

I owe a deep debt of gratitude to **Prof. Dr. RENGARAMANI, M.D., D.G.O.,** Professor of Biochemistry for guiding me to do the dissertation and her constant inspiration given to me throughout this study.

My sincere and special thanks to **Prof. Dr. R. VIJAYAKUMAR, M.D., D.M.,** Professor and Head, Department of Nephrology, Stanley Medical College for permitting me to collect the samples from patients undergoing hemodialysis.

I am very grateful to assistant professors **Dr. R. MAHALAKSHMI, M.D., D.C.H.,** and **Dr. SHANTHI MD, DCP.,** Department of Biochemistry for their valuable guidance and friendly suggestion to carry out this work.

My special thanks to assistant Professor, **Dr. R. SENTHIL KUMAR, M.D.**, **D.C.H.,** Department of Social and Preventive Medicine, and **Mr. A. VENGATESAN, M.Sc., M.Phil., PGDCA**., Lecturer in Statistics, Clinical Epidemiology Unit, Institute of Social Pediatrics, Stanley Medical college Hospital, for their help in statistical work.

I wish to express my special thanks to my colleagues and all the technical staffs of the Department of Biochemistry, Stanley Medical College for their kind cooperation to carry out this work successfully.

I gratefully acknowledge the co-operation rendered by the patients who made it possible for this study to be carried out by me. Without their support this study would not have been possible. I salute the endurance, forbearance and friendly attitude of the patients who took part in this study.

CONTENTS

ABBREVATIONS

1.	GFR	-	Glomerular Filtration Rate
2.	TIBC	-	Total Iron Binding Capacity
3.	ESRD	-	End Stage Renal Disease
4.	RBC	-	Red Blood Cell
5.	CRM	-	Certified Reference Materials.
6.	NaOH	-	Sodium Hydroxide
7.	HCl	-	Hydrochloric Acid
8.	NAD(P)H	-	Reduced Nicotinamide Adenine Dinucleotide Phosphate
9.	Ig G	-	Immunoglobin G
10.	DNA	-	Deoxy Ribonucleic Acid
11.	RNA	-	Ribonucleic Acid
12.	PTH	-	Parathyroid Hormone
13.	APR	-	Acute Phase Reactant
14.	LSD	-	Lysergic acid Diethylamide
15.	NO	-	Nitric Oxide

INTRODUCTION

Chronic Kidney Disease is a process with multiple etiologies, resulting in a continuous decrease of nephron number and function leading to end stage renal disease.

End stage renal disease is a clinical condition in which there is an irreversible loss of renal function rendering the patient to depend permanently on renal replacement therapy (Dialysis or Transplantation).[1]

Chronic kidney disease is the most debilitating condition because of its significant morbidity and mortality. The prevalence of chronic kidney disease is important in planning the care of patients afflicted by it.

The prevalence of chronic renal failure in Indian adult population is 0.78% (~7.85 million Chronic renal failure patients in 1 billion population).[2]

The prevalence of patients with end stage renal disease in India is 120/million population.[3]

The prevalence of patients on dialysis in India is 100/million population.[4]

The National Kidney Foundation / Dialysis Outcome Quality Initiative[5] has defined chronic kidney disease as “either kidney damage (or) GFR <60 mL/min/1.73 m^2 for atleast 3 months”. Kidney damage is defined as

“Pathologic abnormalities or markers of damage including abnormalities in blood (or) urine tests (or) imaging studies.

The goal of hemodialysis in patients with chronic kidney disease is to restore the composition of the internal environment, by diffusional transfer of the solutes along the concentration gradient between the dialysis fluid and blood. This corrects the concentration of various solutes that were initially altered in patients with chronic kidney disease.[6] Dialysis causes changes in the plasma concentration of trace elements in blood, which are the essential nutrients of human beings with a gamut of functions.

The trace elements are components of many enzymes with regulatory, immunologic and antioxidant functions. Trace element disturbances in uremic patients occur due to reduced renal function, proteinuria leading to loss of protein bound elements (Zinc and Copper) and alterations in gastrointestinal absorption. Trace element disturbances in patients on hemodialysis occur depending on the concentration gradient between the ultrafiltrable amount of an element in serum and its concentration in the dialysis fluid. Some trace elements may be removed leading to deficiency where as others present as contaminant in the dialysis fluid could be transferred to patients leading to toxicity.

A survey of yearly statistics of patients undergoing hemodialysis in Nephrology department of Stanley Medical College Hospital shows that the

number has increased significantly over the past 6 years (i.e. 1086 patients in the year 2000 to 2824 patients in the year 2006). As the number of patients on hemodialysis increases, the incidence of complications arising out of it can also increase. Trace element disturbances that are found associated with long term hemodialysis should be treated to improve the quality and longevity of patient's life.

This study was undertaken to evaluate the status of trace elements namely- Zinc, Copper and Iron, and their metabolism in patients, who were on long-term hemodialysis.

AIM AND OBJECTIVE

The aim of this study is to determine the trace element status in patients with end stage renal disease undergoing long-term hemodialysis in the Nephrology department of Stanley Medical College hospital.

The objectives of this study is -

- To determine the indicators of iron metabolism such as serum iron, TIBC, ferritin, transferrin saturation and transferrin in patients on long-term hemodialysis.
- To determine the metabolic indicators of copper – serum copper and ceruloplasmin, and to determine the serum zinc levels in patients on long-term hemodialysis.
- To correlate the relationship between serum iron with copper and zinc in patients on long-term hemodialysis.

REVIEW OF LITERATURE

The kidneys play a central role in homeostatic mechanisms of human body and reduced renal function strongly correlates with increased morbidity and mortality.

Chronic kidney disease is the progressive loss of renal function over months to years leading to end stage renal disease. Without dialysis or transplantation, the mortality associated with end stage renal disease is 100%[7]. Uremia is a syndrome that reflects dysfunction of multiple organs as a result of untreated or undertreated acute or chronic renal failure[1].

EPIDEMIOLOGY:

Worldwide, over 1 million people are alive on dialysis or with a functioning graft[8]. Throughout the world, chronic kidney disease is a growing health problem because of its increasing prevalence, incidence and dismal outcomes.

The US Renal Data System[9] has reported more than 76,500 deaths in patients with end stage renal disease in the year 2001,an annual mortality rate in excess of 20%. This figure has remained unchanged for a decade.

The prevalence of end stage renal disease in South-east Asia is 80-96 cases per million-population[4]. Primary Glomerulonephritis is the most common cause of end stage renal disease worldwide.

Diabetic Nephropathy is a major cause of end stage renal disease and its prevalence in India is 26.8%[4]. Every patient undergoing dialysis has a mean of four co-morbid conditions; about 15 hospital days per year and a self-reported quality of life lower than that of general population[9]. The incidence[11] of various causes of ESRD is given in figure No.1.

RISK FACTORS FOR CHRONIC KIDNEY DISEASE:

The risk factors[1] of chronic kidney disease are as follows.

- Family history of heritable renal disease
- Hypertension
- Diabetes mellitus
- Autoimmune disease
- Past episode of acute renal failure
- Current evidence of kidney damage with normal or even increased glomerular filtration rate

ETIOLOGY OF CHRONIC KIDNEY DISEASE:

- Developmental/congenital conditions:[12]
  - Renal agenesis
  - Aplastic kidneys
  - Renal hypoplasia
  - Ectopic /displaced kidneys
  - Fused kidneys

- Genetic causes (Monogenic inheritance)
    - Polycystic kidney disease
    - Medullary cystic disease
    - Alport's hereditary nephritis
    - Nephronopthisis
    - Fabry's disease
- Neoplasms:
    - Benign and Malignant tumors of kidney
    - Wilm's tumor
- Infections:
    - Recurrent Pyelonephritis
    - Renal Tuberculosis
    - Post- streptococcal glomerulonephritis
- Systemic conditions:
    - Diabetes mellitus
    - Hypertension
    - Gout
    - Hepato-renal syndrome
    - Amyloidosis
    - Scleroderma
    - Good Pasteur syndrome
    - Systemic lupus erythematosis

PATHOPHYSIOLOGY OF CHRONIC KIDNEY DISEASE:

The pathophysiology[1] involves

- Initiating mechanisms specific to the underlying etiology.
- Progressive mechanisms following long term reduction of renal mass, irrespective of etiology, characterized by tubular atrophy, glomerulo-sclerosis, interstitial fibrosis and interstitial mononuclear cellular infiltrates.

The reduction in renal mass is compensated by structural and functional hypertrophy of surviving nephrons. Vasoactive molecules such as cytokines and growth factors mediate this compensatory hypertrophy. It is initially due to adaptive hyperfiltration, mediated by increase in glomerular capillary pressure and flow. The renal function remains relatively normal through these processes, until 75%-80% of the nephrons are damaged and non-functional. These adaptations are short lived, in that they predispose to sclerosis of the remaining viable nephron population. Increased intrarenal activity of renin-angiotensin axis appears to contribute both initial adaptive hyperfiltration and to the subsequent maladaptive hypertrophy and sclerosis.

STAGES OF CHRONIC KIDNEY DISEASE:

Chronic kidney disease is classified into five stages based on glomerular filtration rate and is shown in Table No.1 (Refer Opposite page).

UREMIA:

It is a clinical syndrome that occurs due to profound loss of renal function, resulting in retention of urea and other end products of metabolism, normally excreted in urine. The most common toxins in uremia are the by-products of protein and aminoacid metabolism. The uremic solutes[14] with potential toxicity are given below.

UREMIC TOXINS

- Urea
- Guanidines:
    - Methyl guanidine, Guanidinosuccinic acid, Creatinine, Creatine
- Phenols:
    - O-Cresol, P-Cresol, Benzyl alcohol
- Hippurates:
    - P-hydroxy hippurate
- Phenolic acid:
    - P- hydroxy phenyl acetic acid
- Benzoates
- Polypeptides:
    - Beta-2 micro globulin

- Indoles:
    - Indol-3 acetic acid and Indoxy sulfate
- Middle molecules:
    - Ammonia, Alkaloids, Uric acid, Myoinositol, Oxalate
- Hormones:
    - Parathyroid hormone, Natriuretic factor, Glucagon, Growth hormone, Gastrin
- Xanthine, Hypoxanthine
- Amines:
    - Putrescine, Spermine, Spermidine.
- Endorphins
- Pseudouridine

Uremia involves defective renal excretory, metabolic and endocrine functions. It is accompanied by anemia, malnutrition, impaired metabolism of carbohydrates, fats and proteins, and defective utilization of energy[15]. The clinical abnormalities of uremia are listed below.

CLINICAL ABNORMALITIES IN UREMIA:

The clinical abnormalities[15] in uremia are given below.

1. Fluid and Electrolyte disturbances:
    - Volume expansion and contraction
    - Hypernatremia and hyponatremia
    - Hyperkalemia and hypokalemia

- Metabolic acidosis
- Hyperphosphatemia
- Hypocalcemia

2. Endocrine – metabolic disturbances:

- Secondary hyperparathyroidism
- Carbohydrate intolerance
- Hyperuricemia
- Hypertriglyceridemia
- Protein-calorie malnutrition
- Infertility and sexual dysfunction
- Amenorrhoea
- Hypothermia

3. Neuro muscular disturbances:

- Fatigue
- Headache
- Sleep disturbances
- Peripheral neuropathy

4. Cardiovascular and pulmonary disturbances:

- Congestive cardiac failure (or) Pulmonary edema
- Pericarditis
- Uremic lung

5. Dermatologic disturbances:

- Pallor and Ecchymoses

6. Gastrointestinal disturbances:

- Anorexia, nausea and vomiting
- Gastroenteritis

7. Hematologic and immunologic disturbances:

- Normocytic and normochromic anemia
- Increased susceptibility to infection

RENAL REPLACEMENT THERAPY:

Renal replacement therapy[16] includes dialysis procedures such as

- Hemodialysis
- Peritoneal dialysis
- Continuous hemofiltration
- Continuous hemodiafiltration

Kidney transplantation has become an effective form of renal replacement therapy.

History of Dialysis:

In 1861, Thomas Graham Bell in Glasgow, Scotland, carried out the first dialysis experiments separating crystalloids and colloids in a solution. He coined the term “Dialysis”.[16] The first human dialysis was performed by Georg Haas from Gieben, Germany using large celloidin tubes mounted in glass

containers. In 1943, Willem Kolff at the Groningen University Hospital, introduced the first dialyser suitable for use in man[17].

Definition of Dialysis:

Dialysis is the process of separating macromolecules from ions and low molecular weight compounds in solution, by the difference in their rates of diffusion through a semi-permeable membrane, through which crystalloids can pass readily but colloids pass very slowly (or) not at all[16]. Hemodialysis equipment consists of blood delivery system, the composition and delivery system of the dialysate and the dialyser as shown in figure No.2[18].

Hemodialysis is the most common method used to treat patients with advanced and permanent kidney failure, unsuitable for other modalities of treatment such as peritoneal dialysis and kidney transplantation.

PRINCIPLE OF HEMODIALYSIS:

A constant flow of blood on one side of a semi-permeable membrane and a cleansing solution (dialysate – a fluid with osmotically balanced solution of electrolytes, buffer and glucose in water) on the other, allows removal of waste products by diffusive and convective transport.[19]

The driving force for diffusion is the concentration gradient between the blood and dialysate, which is maintained by countercurrent flows and high flow rates. Excess water from the patient's blood can be removed by manipulating the negative hydrostatic pressure on the dialysate side of the system by a process called Ultra filtration.

COMPLICATIONS OF HEMODIALYSIS:

- Cardiovascular diseases - Coronary artery disease (40%) [20,21]
  Left ventricular hypertrophy (75%)
- Hypertension
- Dialysis Amyloid
- Malnutrition
- Vascular calcification
- Trace element disturbances

A large number of studies have indicated that the concentration of trace elements is altered in patients undergoing hemodialysis.

- Muirhead N, Kertesz A et al[22] had studied the Zinc metabolism in patients on maintanence hemodialysis and evaluated the prevalence of zinc deficiency and abnormalities of zinc metabolism in patients with end stage renal disease.
- Hosokawa S, Nishitani H et al[23] reported serum copper concentration levels in chronic hemodialysis patients. They observed that there was a significant increase in serum copper in patients undergoing hemodialysis, and they found no significant correlation between serum copper and red blood cell count, hematocrit, hemoglobin and serum iron levels.
- Hung KY, Ho CY et al[24] reported trace element burden in geriatric patients and found decreased plasma zinc and increased plasma copper concentrations in hemodialysis patients.

TRACE ELEMENTS

It has been known for long that minerals perform specific functions in the human system and are essential to human beings. They are classified based on their requirement to humans as macro and micro minerals. The micro minerals are also known as trace elements. Trace elements are those that occur in human and animal tissues in mg/kg amounts or less and in µg/dL in body fluids. Ultra trace elements are those that are found at ng/dL (or) µg/kg.[25]

A trace element is considered to be essential, when the signs and symptoms induced by a deficient diet is reversed by an adequate supply of the trace element under investigation. Essential trace elements include Iron, Copper, Zinc, Cobalt, Iodine, Selenium, Molybdenum, Chromium, Boron, Manganese, Nickel, Silicon, Vanadium and Fluorine.

IRON

Iron is the fourth most common element on the planet. It is involved in many important biochemical reactions, and is recognized as an essential nutrient for living organisms. At birth, infants acquire about 350mg of iron from their mothers.[26] This iron is distributed into various compartments in the body. After birth, iron balance is maintained by gastrointestinal absorption from diet.[27,28] There is no physiological mechanism of iron excretion. Obligatory losses (1-2mg/dL) result from shedding of epithelial cells from

intestinal and urinary tracts, from desquamation of skin, and from menstruation by women in their child-bearing years.[28,29]

SOURCES:

Heme and non-heme iron from animal and plant sources respectively, are the two main sources of iron .The foods rich in heme and non-heme iron are enumerated in Table No.2 (Refer Opposite Page).

DAILY REQUIREMENTS OF IRON:

The recommended daily intake of iron is given in Table No.2(a) (Refer Opposite page).

ABSORPTION:

Iron is absorbed mainly in the enterocytes of duodenum and proximal jejunum as shown in Figure No.3[30]. Of the iron absorbed, 25% is heme iron and only 5% is non-heme iron. Non-heme iron present mostly in ferric form is ionized by gastric juice and reduced by Ferrireductase to the more soluble ferrous state in which form iron is absorbed. Vitamin C in food also favors reduction of ferric iron to ferrous iron. The transfer of iron from the apical surfaces of enterocytes into their interior is performed by a proton-coupled divalent metal transporter (DMT 1). Heme is transported into the enterocytes by heme transporter (HT), and heme oxidase (HO) releases Fe^{2+} from the heme. Inside the enterocytes, iron can either be stored as ferritin or transferred across the basolateral membrane by another protein, iron regulatory protein (IREG1) (or) ferroportin –1 into the plasma, where it is carried by transferrin. Ferroportin-1 interacts with the copper-containing protein hephaestin (HP),

which is similar to ceruloplasmin, having ferroxidase activity that oxidizes ferrous ion to ferric ion, the form in which iron is transported in the plasma by transferrin to other tissues. Regulation of iron balance involves a 25-aminoacid peptide, Hepcidin (Hep – hepatocyte, Cidin – bactericidal protein), synthesized in the liver. Hepcidin synthesis increases in response to iron overload and is decreased in iron deficiency. Hepcidin binds ferroportin-1 and induces its internalization and lysosomal degradation preventing iron efflux from enterocytes and reticuloendothelial / hepatic macrophages to the plasma[31].

The compartments of iron in our body are given in Table No.3 (Refer Opposite Page).

TRANSFERRIN:

A plasma iron transport protein - apotransferrin, binds the iron absorbed from the intestine or released from catabolism of hemoglobin. This iron is then transported to storage sites such as liver and reticuloendothelial system, and to erythropoietic tissue for hemoglobin synthesis.

Apotransferrin is a β_1 – globulin with a molecular of 75,000 Da, having two binding sites for ferric iron per molecule. The apotransferrin-Fe^{3+} complex is called transferrin.

Transferrin consists of a single polypeptide chain and two homologous domains, each containing a Fe^{3+} binding site. Transferrin is synthesized mainly in liver and choroid plexus of brain. Transferrin has a half-life of about 8 – 10 days. Transferrin reversibly binds many polycations - iron, copper, zinc, cobalt

and calcium. Based on the transferrin concentration of 3mg/mL of plasma, the total transferrin bound iron in the body is about 3mg. (Table No.3)

IRON UPTAKE BY CELLS:

Transferrin is recognized by specific cell membrane transferrin receptors present in all nucleated cells especially in erythroid precursors, placenta, liver, neoplastic tissue, and rapidly dividing normal cells.[33] The receptor can bind two molecules of transferrin. The affinity of receptor for its ligand depends on both the iron content of transferrin and the pH. Transferrin binds to the receptors on the cell membrane and is internalized enclosed within the endocytic vesicles. Iron is then released from transferrin due to acid pH inside the vesicle. Once released, ferric form is reduced to ferrous form and incorporated into ferritin and hemosiderin, and used for the synthesis of compounds such as, hemoglobin, myoglobin and cytochromes. The receptor – apotransferrin complex is recycled to cell surface where the apotransferrin is released and recycled, making the receptor available again for binding.

STORAGE IRON:

Iron is stored in our body in the form of ferritin and hemosiderin. In hepatocytes, macrophages of bone marrow and other organs, ferritin provides a reserve of iron readily available for formation of hemoglobin and other heme proteins. Thus iron is shielded from body fluids, so that it is unable to produce oxidative damage, as would be the case if it were in ionic form.

Ferritin consists of an apoferritin shell and an interior ferric-oxyhydroxide $(FeOOH)_X$ crystalline core. The apoferritin shell consists of 24 subunits, which are either L (light) or H (heavy) ferritin chains. The proportion of light to heavy chains differs from tissue to tissue as shown in figure No.4.[34]

Only ferrous iron is taken up by ferritin, and a catalytic site on the H chain oxidizes it to ferric form. Iron is released from ferritin nonenzymatically by reduced flavin mononucleotide and other reducing substances.

Ferritin is present in the blood in very low concentration. The circulating form differs from tissue ferritin in that it is glycosylated, contains mostly L chains, and is poor in iron, representing mostly apoferritin. The plasma ferritin concentration declines very early in the development of iron deficiency, long before changes are observed in blood hemoglobin concentration, red blood cell size and serum iron concentration.

HEMOSIDERIN:

Hemosiderin is an amorphous water-soluble compound formed by aggregated and partial deproteinised ferritin. It is found predominantly in cells of the liver, spleen and bone marrow. Iron is released slowly from hemosiderin because it occurs in relatively large aggregates and therefore has a much smaller surface/volume ratio.

CLINICAL SIGNIFICANCE OF SERUM IRON, TIBC, TRANSFERRIN, TRANSFERRIN SATURATION AND FERRITIN:

SERUM IRON:

The serum iron concentration refers to the iron bound to transferrin and does not include the iron in free hemoglobin. There is a diurnal variation with a fall in iron concentration in evening. Significant day-to-day variation occurs as well[35]. The conditions that affect serum iron concentration have been enlisted in the Table No.4 (Refer Opposite Page). Moreover for proper interpretation of serum iron, the levels of total iron binding capacity (TIBC) and transferrin saturation are also necessary.

TOTAL IRON BINDING CAPACITY:

Normally only one third of transferrin is bound with iron. Thus serum transferrin has considerable reserve iron binding capacity. This is called the serum unsaturated iron binding capacity (UIBC). The TIBC is a measurement of maximum concentration of iron that transferrin can bind and is therefore an indirect way of assessing transferrin level. Serum transferrin concentration can be estimated from TIBC by the following relationship[36].

$$\text{Serum transferrin (mg/dL)} = 0.70 \times \text{TIBC (}\mu\text{g/dL)}$$

(or)

$$\text{TIBC (}\mu\text{g/dL)} = 1.43 \times \text{Transferrin (mg/ dL)}$$

The clinical significance of Serum transferrin and TIBC are given in Table No.5 (Refer Opposite Page).

SERUM TRANSFERRIN SATURATION:

Transferrin saturation indicates the iron readily available for erythropoiesis. Transferrin saturation is calculated from serum iron and TIBC as follows[37].

$$\text{Transferrin Saturation (\%)} = \frac{\text{Serum iron}}{\text{TIBC}} \times 100$$

Clinical conditions associated with changes in transferrin saturation are enlisted in Table No.6 (Refer Opposite Page).

SERUM FERRITIN:

The measurement of serum ferritin provides the most useful indirect estimate of the body iron stores.[38,39] However ferritin is an acute phase reactant[37]. Hence in fever, acute infections, rheumatoid arthritis and chronic inflammatory disorders, ferritin is found to be elevated. The clinical conditions associated with changes in serum ferritin are enumerated in the Table No.7 (Refer Opposite Page).

Among the biochemical markers mentioned above, serum ferritin has been found to be the most reliable parameter for assessment of body iron status as it is least influenced by extraneous factors. [38,39,40,41]

IRON DEFICIENCY IN CHRONIC KIDNEY DISEASE PATIENTS ON HEMODIALYSIS:

Currently, the 2 best tests of iron status of chronic kidney disease patients on hemodialysis are percent transferrin saturation and serum ferritin. In normal healthy persons, iron deficiency is considered absolute, when iron stores are depleted, as indicated by serum ferritin <12 ng/mL, and iron delivery to erythroid marrow is impaired, as evidenced by transferrin saturation <15%.

Absolute iron deficiency in chronic kidney disease patients has been defined as serum ferritin <100 ng/mL and transferrin saturation <20%. Iron deficiency is common in patients on hemodialysis due to blood loss in the dialyser tubing and the dialyser, frequent blood sampling and gastrointestinal blood losses that cannot be compensated for by sufficient absorption of iron from the gastrointestinal tract.[42]

ZINC

Zinc is the second most abundant trace element in the body next to iron. Zn^{2+} with atomic number 30 and atomic weight 65.39 has a filled 3d electron shell and is a stable ion. Zinc is a good electron acceptor (strong Lewis acid) with no redox reactions. Zinc is known to be essential for growth and development of all organisms.

DIETARY SOURCES:

Zinc is widely distributed in foods mainly bound to proteins. Red meat and fish are rich in zinc. Wheat germ and whole bran are good sources but milling and food processing reduce their zinc content.

DAILY REQUIREMENTS:

The daily requirements of zinc are given in the Table No.8 (Refer Opposite Page).

ABSORPTION, TRANSPORT AND METABOLISM:

About 30 % ± 10 % of dietary zinc is absorbed in the small intestine, as estimated by double isotopic tracer ratio method.[44] Absorption of Zn in the small intestine is decreased by phytates, phosphates, calcium, iron, copper, dietary fiber and a constituent of beans.[45,46] Absorption is increased by glucose, aminoacids and peptides .

Absorbed zinc is transported to the liver by portal circulation, where active incorporation into metalloenzymes and plasma proteins occurs.

In the circulation, zinc is 80% bound to albumin and most of the rest is bound to alpha-2 macroglobulin.[47]

Total adult body zinc is 2 to 2.5g and the metal is present in cells of all metabolically active tissues and organs. About 55% of the total is found in muscle and about 30% in bone.[45] The prostate, semen and retina have high local concentration of zinc. In RBC's, zinc in the form of carbonic anhydrase is about 10 times more concentrated than plasma.

Zinc is excreted in both feces and urine. Fecal excretion includes both unabsorbed dietary zinc and zinc resecreted into the gut from pancreatic fluid and other intestinal fluids.[48] Urinary excretion of zinc is about 0.5 mg/day. Zinc excretion is markedly increased in catabolic process such as post-operative states and in starvation as a result of release from skeletal muscle.[49]

FUNCTIONS OF ZINC:

Zinc is an essential constituent of all six classes of enzymes as well as transcription and replication factors.[50,51] Important examples include carbonic anhydrase, alkaline phosphatase, alcohol dehydrogenase, RNA and DNA polymerase, thymidine kinase and superoxide dismutase.[52] Zinc fingers[53,54] are biologically active molecules that play a role in gene expression by acting as DNA binding transcription factors and play a key role in developmental

biology and also in the regulation of steroid, thyroid and other hormone synthesis.

Zinc is essential for normal spermatogenesis, sperm physiology, and normal embryonic development and is essential for formation and function of the immune system. Zinc also plays a role in the sense of taste and in wound healing.[55]

DEFICIENCY OF ZINC:

Fasting morning plasma zinc less than 30 μg/dL suggests likely deficiency of zinc[56]. The clinical presentation of deficiency disease is varied, non-specific, and related to the degree and duration of the depletion.[48,57,58] Disorders of zinc deficiency includes growth retardation, alopecia, acrodermatitis, diarrhoea, immunologic dysfunction, failure to thrive, psychological disturbances, gonadal atrophy, impaired spermatogenesis and congenital malformations.[55]

ZINC IN RENAL FAILURE:

Urinary zinc excretion varies from 375±150 μg/dL to 643±198 μg/dL. The zincuria is due either to displacement from protein or to competition between zinc and the aminoacids for tubular reabsorption.

Patients with renal failure ingesting low protein diet and with marked proteinuria develop low plasma zinc levels. Patients undergoing hemodialysis

on ferrous sulphate tablets results in malabsorption of zinc. Some hemodialysis patients have high zinc levels due to the use of either zinc oxide plasters that bind the coils of artificial kidney, or to higher zinc content in the dialysate as a result of galvanized iron used in hemodialysis tank.[59]

LABORATORY ASSESSMENT OF ZINC STATUS

PLASMA ZINC:

Plasma zinc concentrations exhibit both circadian and postprandial fluctuations. Zinc levels decrease after food and are higher in the morning than in the evening. Serum zinc levels are 5% to 15% higher than plasma levels because of osmotic fluids shift from the blood cells when various anticoagulants are used.[56]

COPPER

Copper was one of the first metals known to man, used probably between 8000 and 7000 BC. The word copper derives from the Latin 'Cuprum', an altered version of 'cyprium'. The original name of copper is 'aescyprium', refers to the island of Cyprus where the ancient copper mines were located.[60]

Copper is a transition metal with atomic number 29 and an atomic weight of 63.55 Daltons. Copper participates in metabolism as a component of many metalloenzymes, including ceruloplasmin or ferroxidase I, cytochrome- c oxidase, copper/zinc superoxide dismutase, dopamine beta-hydroxylase, tyrosinase, monoamine oxidase, Diamine oxidase, lysyl oxidase (protein-lysine 6-oxidase) and ferroxidase II.

DIETARY SOURCES:

The richest dietary sources of copper include nuts, seeds, legumes, and the bran and germ portions of grains, liver, kidneys, shellfish, oysters and crustaceans. Cow's milk has little copper.

DAILY REQUIREMENTS OF COPPER:

World Health Organisation estimated minimum daily requirements[61] of copper is

Male : 0.6 mg/day

Female : 0.7 mg/day

ABSORPTION, TRANSPORT AND METABOLISM

Copper is principally absorbed in small intestine as well as in the stomach and its absorption is reduced by zinc (via metallothionein), molybdate and iron and is increased by aminoacids and dietary sodium.[62]

Absorbed copper is transported to liver in portal blood bound to albumin, where it is incorporated by hepatocytes into cuproenzymes and proteins and then exported to peripheral tissues. More than 90% copper exported from liver is in the form of glycoprotein - ceruloplasmin. A smaller amount of copper (10%) is transported in plasma bound to albumin and this copper is in equilibrium with plasma aminoacids. This fraction is essential for cellular uptake.

About 0.5 to 2 mg of copper is excreted via bile into feces daily. Copper losses in urine and sweat are < 3% of dietary intake. Urine copper output is normally < 60 μg/day.[63]

FUNCTIONS OF COPPER:

Copper is essential for intracellular energy production, connective tissue formation, metabolism of iron (ceruloplasmin oxidizes ferrous to ferric ion and incorporates iron into transferrin and eventually into hemoglobin), synthesis of melanin, antioxidant function and regulation of gene expression.[63]

COPPER DEFICIENCY:

Copper levels in adults below 50μg/dL and for infants below 30μg/dL indicates copper deficiency.[63]

INDIVIDUALS AT RISK FOR COPPER DEFICIENCY:

Individuals at risk for copper deficiency are given below.[63, 64, 65, 66, 67]

- Malnourished infants
- Premature infants
- Patients on total parenteral nutrition and prolonged enteral feeding via jejunostomy
- Menke's syndrome
- Malabsorption syndrome-celiac disease, tropical sprue, cystic fibrosis cardiovascular disease

COPPER TOXICITY:

Toxicity arises from copper contamination of diet and water supplies. Acute poisoning occurs with accidental (or) intentional ingestion of copper sulphate. Wilson disease is a genetic disorder of copper metabolism that causes an increase in copper to toxic levels.[68,69]

COPPER IN RENAL FAILURE:

Plasma copper levels in chronic kidney disease patients are usually normal but could be lower than normal. In patients with proteinuria, urinary copper concentration increases, as it is lost along with bound proteins that lead to low plasma copper.[59]

LABORATORY ASSESSMENT OF COPPER:

Plasma copper and ceruloplasmin assays are widely used to confirm copper deficiency. As 90% of copper is bound to ceruloplasmin, and factors that increase hepatic synthesis of ceruloplasmin, such as an acute phase reaction or the oral contraceptive pill, will increase plasma copper independently of dietary copper intake.[70]

CERULOPLASMIN

Ceruloplasmin (Molecular mass 132 KDa) is an alpha-2 globulin that contains approximately 95% of the total copper found in serum. Each molecule of ceruloplasmin contains 6 to 8 copper atoms. The high content of copper gives ceruloplasmin, a blue color. Ceruloplasmin also binds magnesium.

Ceruloplasmin has single polypeptide chain with 1046 aminoacids and three glucosamine - linked oligosaccharide side chains, with a total carbohydrate content of 8% to 9.5%. Ceruloplasmin is synthesized in hepatic parenchymal cells, with a small amount synthesized in macrophages and lymphocytes. The normal plasma half-life of intact, holoceruloplasmin (copper replete) is 4 – 5 days, whereas that of apoceruloplasmin is few hours.[71] The structure and functional sites of human ceruloplasmin is given in figure No.5.

FUNCTIONS OF CERULOPLASMIN:

The primary role of ceruloplasmin is that, it functions as an oxidant (or) anti-oxidant depending on factors such as presence of free ferric ions and ferritin binding sites. Ceruloplasmin is vitally important in the regulation of ionic state of iron (oxidizing ferrous to ferric ion and permitting its incorporation into transferrin without formation of toxic iron products).

Ceruloplasmin transport small amounts of copper to tissues, which has separate membrane receptors for ceruloplasmin and albumin bound copper.

CLINICAL SIGNIFICANCE OF CERULOPLASMIN:

- **Increased plasma levels:**
  - Primary cause : Genetic
  - Secondary cause : Acute phase reactions, pregnancy and estrogen therapy
- **Decreased plasma levels:**
  - Primary cause : Genetic deficiency
  - Secondary cause : Dietary copper insufficiency (Malabsorption)
    - Menke's disease
    - Wilson's disease[72]

LABORATORY ASSESSMENT:

Ceruloplasmin is assayed immunochemically or functionally (copper oxidase activity). Immunochemical methods measure both the intact molecule and apoceruloplasmin and proteolytic fragments. Copper oxidase method measures only the native copper containing ceruloplasmin.

Serum (or) plasma is preferred. After collection, the samples should be centrifuged as soon as possible to avoid spontaneous oxidation invitro.[72]

CERULOPLASMIN IN RENAL FAILURE:

The urinary loss of ceruloplasmin is increased in proteinuria that results in decreased plasma levels of ceruloplasmin.[59]

MATERIALS AND METHODS

This is an age and sex matched comparative study. The present study is conducted after getting approval from the ethical committee of Stanley Medical College, Chennai. The study was conducted on 100 subjects that include two groups. Group 1 consists of 50 normal subjects and group 2 consists of 50 end stage renal disease patients on hemodialysis 2 times/week, between age group 10 years and 74 years. Group 1 serves as control for group 2.

For group 1, the subjects were volunteers from the patient's relatives selected on the basis of good health as evidenced by medical history, complete physical examination and routine laboratory tests performed before the commencement of study.

Group 2 patients were selected from those attending the Nephrology department of Stanley Medical College Hospital from April' 07 to July' 07. The study subjects were clearly informed of the nature of the study and the samples were collected after getting written informed consent. The samples were analyzed for iron, ferritin, total iron binding capacity (TIBC), transferrin saturation, transferrin, zinc, copper, ceruloplasmin and the results were analyzed based on the data collected.

INCLUSION CRITERIA:

- Patients with end stage renal disease undergoing hemodialysis 2 times per week
- Age 10 years to 74 years

EXCLUSION CRITERIA:

- Patients receiving parenteral iron supplements within 3 months of study and drugs such as aspirin, allopurinol, metformin, glucocorticoids and oral contraceptive pills.
- Patients receiving recombinant human erythropoietin within 3 months of study.
- History of recent blood transfusion.
- History of bleeding episodes.
- Patients with infection and inflammatory conditions like rheumatoid arthritis.
- Patients with hepatocellular damage.
- Patients with malignancies such as leukemia, lymphoma, breast carcinoma.
- Patients not willing to give written informed consent were excluded from the study.

SAMPLE COLLECTION:

Venous blood 10 mL was collected just before dialysis from each subject. After collection, blood samples were centrifuged to separate serum.

Biochemical analyses were carried out immediately after collection. In case of delay, samples were stored at ─20°C. All the chemicals used were of analytical reagent grade.

DETERMINATION OF TRACE ELEMENTS IN SERUM:

1.SERUM IRON: (µg/dL)

Serum iron was estimated by the Ferrozine method[73] of White J.M., and Flashka, H.A.

PRINCIPLE:

Serum is treated with buffer to prevent precipitation of proteins and to provide an acid medium to dissociate ferric-transferrin complex and to reduce ferric to ferrous ion. Addition of color reagent forms a deeply colored ferrozine-iron complex with maximum absorbance at 562nm.

STANDARDISATION OF THE PROCEDURE:

Preparation of standards: Ferrous sulphate ($FeSO_4.7H_2O$) was employed for preparation of iron standards.

STOCK SOLUTION: 10 mg/dL of Iron

Dissolve 490 mg of $FeSO_4.\ 7H_2O$ (British Drug House Ltd) in deionised water and add 1 mL of concentrated sulphuric acid and make upto 1litre with deionised water.

WORKING STANDARDS:

Working standards of various concentrations were prepared from the stock solution as shown in Table No.10 (Refer Opposite Page).

REAGENTS:

1. Ferrozine color reagent:

400 mg of Ferrozine (Hi Media) and 2.5 g of Thiourea (Indian Drug and Pharmaceuticals) is dissolved in 100 mL of deionised water. Filtered and stored in a dark color bottle. The reagent is stable for two months.

2. NaOH 12.5 mol/L:

50 g of NaOH (Indian Drug and Pharmaceuticals) is dissolved in 100 mL of deionised water and stored in polyethylene bottle.

3. Stock acetate buffer 1 mol/L:

60mL of glacial acetic acid dissolved in 28 mL of 12.5 M NaOH and made up to 500 mL with deionised water. The pH of the buffer is adjusted with acetic acid (or) NaOH to 4.5.

4. Iron reagent-A:

6g of Sodium Lauryl Sulphate (Hi Media) dissolved in 10 mL of Stock acetate buffer and made up to 100 mL with deionised water. The solution is stable for 4 weeks.

5. Iron reagent B:

6 g of Ascorbic acid (Hi Media) and 1g of Sodium metabisulfite (Indian Drug and Pharmaceuticals) dissolved in 10 mL of stock acetate buffer and made upto 100 mL with deionised water. This solution is stored in dark brown bottle in the refrigerator and is stable for 2 weeks.

6. Iron Buffer Reagent:

Equal volumes of Iron reagent A and B are mixed and prepared freshly.

TABLE NO.11

PROCEDURE

	Test	Standard	Blank
Sample	0.5 mL	-	-
Standard	-	0.5mL	-
Deionised water	-	-	0.5mL
Iron buffer reagent	4.5mL	4.5mL	4.5mL

Mix vigorously and incubate for 15 min at 37˚C in a water bath. Read the absorbance (A_1) at 562 nm.

0.1 mL of Ferrozine reagent is then added to all tubes and incubated for 15 min in a water bath at 37˚C.Read the absorbance (A_2) at 562 nm.

ABSORBANCE OF IRON STANDARDS:

Reagent blank = 0.187

TABLE NO.12

Concentration of Working Standard (µg/dL)	Standard Absorbance	Standard – Blank Absorbance
50	0.202	0.015
100	0.215	0.028
200	0.242	0.055
300	0.272	0.085
400	0.308	0.121

CALCULATION:

$$\text{Iron } (\mu g/dL) = \frac{\text{A2-A1 (Unknown)}}{\text{A2-A1 (Standard)}} \times \text{Concentration of Standard}$$

REFERENCE VALUES:

Adults

Male : 60 - 150 µg/dL

Female: 50 – 130 µg/dL[74]

2. SERUM TOTAL IRON BINDING CAPACITY (µg/dL):

TIBC was determined using Magnesium Carbonate absorption method[75] of Ramsay, 1957,1958.

PRINCIPLE:

An excess of iron is added in the form of ferric chloride to serum to saturate transferrin. The excess unbound iron is then precipitated with basic magnesium carbonate. After centrifugation, the iron in the supernatant is determined by Ferrozine method colorimetrically at 562 nm.

REAGENTS:

1. **Ferric chloride Solution**: 5µg iron per mL in 5 mmol/L HCl. Stock solution containing 144 mg of anhydrous ferric chloride (Merck) in 100 ml of 500mM HCl was prepared. The stock solution was diluted 1 in 100 with deionised water to obtain a concentration of 5 µg of iron per mL in hydrochloric acid (5mmol).

2. **Light magnesium carbonate powder** (Lobachem)

PROCEDURE:

1. One volume of serum is mixed with 3 volumes of ferric chloride solution and is covered.
2. After 5 min, light magnesium carbonate powder (100 mg/mL of iron solution) was added and covered with parafilm .The solution was mixed continuously at 10 min intervals for upto 30 min.
3. The solution was then centrifuged for 10 min and the supernatant was analyzed for iron using Ferrozine method.
4. The result must be multiplied by factor 3 (Dilution factor for ferric chloride solution).

REFERENCE INTERVAL FOR TIBC:

Adults : 270 – 380 µg/dL

Reference interval for methods not requiring protein precipitation:

Adults : 280 - 400 µg/dL [74]

3. SERUM FERRITIN:(ng/mL)

Serum ferritin was determined by micro plate immuno enzymometric sequential assay –ACCUBIND FERRITIN MICROPLATE ELISA KIT (LILLAC).

PRINCIPLE:

Monoclonal biotinylated antibody and serum containing ferritin antigen is added to micro plate wells coated with streptavidin to form an immobile complex. After that another antibody labeled with enzyme is added that interacts with the immobile complex to form an Antibody-Antigen-Biotinylated Antibody complex on the surface of well. When a substrate is added, it produces a color which is measured at 450nm using a microplate spectrophotometer.

STANDARDS:

Ferritin calibrators of 6 concentrations were provided with the kit. The given calibrators were

0 ng/mL, 10 ng/mL, 50 ng/mL, 150 ng/mL, 400ng/mL, 800ng/mL.

CALIBRATION:

The absorbance of the calibrators (Table No.13) is plotted against the concentration of ferritin on a graph paper as shown in graph no.2. The concentration of ferritin for an unknown is determined by plotting the absorbance of unknown on the vertical axis of the graph and reading the concentration (ng/mL) from the horizontal axis of the graph.

REAGENTS:

1. **Biotinylated monoclonal mouse IgG** in buffer, dye and preservative. Store at 2˚ – 8˚ C
2. **Horseradish peroxidase labeled anti – ferritin IgG antibody** in buffer, dye and preservatives. Store at 2˚ – 8˚ C
3. **Streptavidin coated micro plate** – 96 wells. Store at 2˚ – 8˚ C
4. Wash Solution Concentrate containing a surfactant in buffered saline. The wash concentrate was diluted in 1 litre of deionised water for use. Store at 2˚ – 30˚ C
5. Substrate A containing **Tetra methyl benzidine (TMB**) in buffer. Store at 2˚ – 8˚ C
6. Substrate B Containing **hydrogen peroxide** in buffer. Store at 2˚ – 8˚ C
7. Substrate A and Substrate B were mixed in equal proportions for use. Store at 2˚ – 8˚ C
8. Stop solution containing strong acid (**1N HCl**). Store at 2˚ – 30˚ C

PROCEDURE:

1. 25µL of calibrators were pipetted into first 6 wells and the samples into the remaining wells.
2. 0.1 mL of Biotinylated antibody was then added to each well.
3. After swirling the plate for 20 – 30 sec, the plate was incubated for 30 minutes.

4. Then the plate was washed 3 times with 300µL of wash buffer and decanted.
5. 0.1 mL of anti – ferritin enzyme labeled antibody was then added.
6. The plate was incubated for 30 min after gentle swirling.
7. The plate was again washed 3 times with 300 µL of wash buffer and decanted.
8. 0.1 mL of substrate was added and incubated for 15 min. Then 0.05 mL of stop solution was added and the plate was mixed and the absorbance was read at 450 nm. (Using a reference wavelength of 620 – 630 nm)

VALIDITY OF THE ASSAY:

Maximum Absorbance (800 ng/mL calibrator) ≥ 1.2

Maximum Absorbance (0 ng/mL calibrator) ≤ 0.1

TABLE NO.13

ABSORBANCE OF FERRITIN CALIBRATORS

Concentration of Standard (ng/mL)	Absorbance of Ferritin
0	0.01
10	0.11
50	0.58
150	1.14
400	1.51
800	1.86

REFERENCE RANGE:

Adult male : 20 – 250 ng/mL

Adult Female : 10 – 120 ng/mL [76]

ABSOLUTE IRON DEFICIENCY IN HEMODIALYSIS PATIENTS:

Serum Ferritin < 100 ng/mL [42]

4. SERUM TRANSFERRIN: (g/L)

Calculated from Total iron binding capacity.[36]

Serum Transferrin (g/L) = 0.007 x TIBC (µg/dL)

Derivation of factor:

Theoretical ratio of TIBC (µmol/L) to transferrin (g/L) is 25.1[77,78]

∴ TIBC (µmol/L) = 25.1 x Transferrin (g/L)

1 mol of Iron = 55.8 g/L

1µmol = 55.8 µg/L

∴ 1 µg/L = 1/ 55.8

1 µg/L = 0.0179 µmol

TIBC (µg/L) = 25.1/0.0179 x Transferrin (g/L)

∴ Transferrin (g/L) = 0.007 x TIBC (µg/dL)

The reference interval for serum transferrin is given in Table No.14 (Refer Opposite Page).

5. TRANSFERRIN SATURATION: (%)

Serum transferrin saturation [37] is determined as follows,

$$\text{Transferrin Saturation} = \frac{\text{Serum Iron}}{\text{TIBC}} \times 100$$

REFERENCE RANGE :

Male : 20 – 55 %

Female: 15 –50 % [80]

ABSOLUTE IRON DEFICIENCY IN HEMODIALYSIS PATIENTS:

Serum Transferrin Saturation <20 % [42]

6. SERUM ZINC: (µg/dL)

Zinc was measured Colorimetrically with 5-Brom-PAPS [GREINER COMMERCIAL KIT].

PRINCIPLE:

Zinc forms red chelate complex with 2-(5-Bromo-2-Pyridylazo)-5-(N-Propyl-N-Sulfopropylamino)-Phenol. The increase in absorbance is measured at 560nm and is proportional to concentration of total zinc in the sample.

STANDARD PREPARATION:

The standard provided with the kit was 200 µg/dL of Zinc.

Working standards were prepared from the given concentration as shown in Table No.15 (Refer Opposite Page).

REAGENTS:

1. **Monoreagent**(ready to use)

1.	5-Brom-PAPS	=	0.02 mmol
2.	Bicarbonate buffer pH 9.8	=	200 mmol
3.	Sodium Citrate	=	170 mmol
4.	Dimethyl glyoxime	=	4 mmol
5.	Detergent	=	1%

The reagent is stable when it is stored at 2˚ – 25˚ C

PROCEDURE:

Wavelength: 560 nm

Light Path: 1cm

Temperature: 25˚C/37˚C

TABLE NO.16

	Standard	**Test**	**Reagent blank**
Reagent	1000µL	1000µL	1000µL
Sample	-	50µL	-
Standard	50µL	-	-

Mix and incubate for 10 min at 25˚ C (or) 5 min at 37˚ C. Read the absorbance at 560 nm.

TABLE NO.17

ABSORBANCE OF ZINC STANDARDS

REAGENT BLANK = 0.105

Concentration Of Zinc (µg /dL)	Standard Absorbance	Standard – Blank Absorbance
50	0.166	0.061
100	0.227	0.122
200	0.345	0.240

CALCULATION:

$$\text{Zinc (µg/dL)} = \frac{\text{Test absorbance}}{\text{Standard absorbance}} \times \text{Concentration of Standard}$$

REFERENCE RANGE:

Serum Zinc : 70 – 150 µg/dL[81]

7. SERUM COPPER: (µg/dL)

Serum Copper was measured using Diethyldithiocarbamate method [82] of Eden and Green, 1940; Ventura.S and King E. J.1951.

PRINCIPLE:

After releasing copper from protein by hydrochloric acid, the proteins were precipitated by trichloroacetic acid, and the copper extracted into mixture

of amyl alcohol and ether as a golden yellow colored complex with sodium diethyldithiocarbamate for colorimetric determination at 440nm.

STANDARDISATION:

Copper sulphate ($CuSO_4.5H_2O$) was used to prepare the standard solutions.

STOCK SOLUTION: 10 mg/dL.

To 393 mg of $CuSO_4.5H_2O$ (chemspure co.), 1 mL of Concentrated Sulphuric acid was added and made to 1 litre with deionised water.

Working standards were prepared from 10 mg% of stock solution as shown in Table No.18 (Refer Opposite Page).

REAGENTS:

1. Hydrochloric acid, 100 mmol/L
2. Hydrochloric acid, 6 mol/L
3. Trichloroacetic acid (TCA) solutions, 200 g/L and 50 g/L (Qualigens)
4. Sodium pyrophosphate solution, 200 g/L (S.D.Fine Chemicals)
5. Ammonia, Sp.gr. 0.88 (Merck)
6. Sodium diethyldithiocarbamate solution, 4 g/L (British Drug House Ltd)
7. Amyl alcohol (Ranbaxy Laboratories) - Ether (Hi Media) mixture containing equal volumes each.
8. Powdered anhydrous Sodium Sulphate. (Hi Media)

PROCEDURE:

1. To 3ml of serum, 1mL of 100mmol/L HCl was added and warmed until the solution turns cloud.
2. Cool and 1.5 mL of 6 mmol/L HCl was added and allowed to stand for 10 min.
3. 3 mL of 200g/L TCA was added and allowed to stand for few minutes and centrifuged to separate the supernatant.
4. 3 mL of 50 g/L TCA was then added to wash the precipitate and the supernatant fluids were combined.
5. 1 mL of sodium pyrophosphate, 2 mL of ammonia, and 1 mL of sodium diethyldithiocarbamate was then added.
6. Shake the solution with 5 mL amyl alcohol and ether mixture for 2 min to extract copper.
7. The organic layer was removed and dried with anhydrous sodium sulphate.
8. The absorbance was read using violet filter (440 nm).

TABLE NO.19

ABSORBANCE OF COPPER STANDARDS

REAGENT BLANK= 0.028

Concentration of Standard (µg/dL)	Standard absorbance	Standard - Blank Absorbance
50	0.070	0.042
100	0.113	0.085
200	0.194	0.166
300	0.270	0.242
400	0.381	0.353

CALCULATION:

$$\text{Copper (µg/dL)} = \frac{\text{Test absorbance – Blank absorbance}}{\text{Standard absorbance – Blank absorbance}} \times \text{Concentration of Standard}$$

REFERENCE INTERVAL:

Birth to 6 months : 20 –70 µg/dL

6 months - 6 years : 90 – 190 µg/dL

6 years – 12 years : 80 - 160 µg/dL

Adult Male : 70 – 140 µg /dL

Adult Female : 80 – 155 µg /dL [83]

8. CERULOPLASMIN: (UNITS)

Ceruloplasmin was determined by Copper Oxidase activity using Para-phenylenediamine by two-point procedure of Henry[84] in spectrophotometer.

PRINCIPLE:

Ceruloplasmin catalyses the oxidation of Paraphenylenediamine hydrochloride to give dark lavender color product which is measured at 530nm.

REAGENTS:

1. **Acetate Buffer**. PH 6.0 (0.1 M)

   10 mL of 0.1 M acetic acid (0.57 mL of glacial acetic acid made upto 100 mL) was added to 200 mL of 0.1 M Sodium acetate (0.82 g 0f anhydrous sodium acetate in 100 mL of water). Adjust the pH using 0.1M acetic acid (or) sodium acetate.

2. **Sodium Azide** : 0.1%

   0.1 g of Sodium azide (S.D Fine chemicals) in 100 mL of 0.1 M acetate buffer.

3. **Paraphenylene Diamine** : 0.25 %

   0.25 g of paraphenylene Diamine (Hi Media) in 100 mL of 0.1 M acetate buffer

TABLE NO.20

PROCEDURE

Reagents	**Blank**	**Test**
Acetate Butter	1ml	2ml
0.25% Paraphenylene Diamine in Acetate buffer	1ml	1ml
Sodium Azide	1ml	-

Bring the test solutions to 37°C by placing in water bath.

Serum	0.1ml	0.1ml

After 10 and 40 min of incubation at 37°C, read the absorbance at 10 min and 40 min interval at 540nm.

CALCULATION:

$$\text{Ceruloplasmin (Units)} = \frac{(T - B)}{(40\ \text{MIN})} - \frac{(T - B)}{(10\ \text{MIN})} \times 1000$$

REFERENCE INTERVAL:

Normal Range = 250 – 570 Units [84]

9. BLOOD UREA: (mg/dL)

Blood Urea was determined by enzymatic method using BUN – Glutamate Dehydrogenase method[85] in Micro 200 Semi autoanalyser at 340nm.

PRINCIPLE:

Urea in the presence of urease forms ammonia, which reacts with 2-oxoglutarate and NAD(P)H in the presence of glutamate dehydrogenase to form NAD(P) and glutamate. The decrease in the absorbance of NAD(P)H is measured at 340nm.

REFERENCE INTERVAL:

New Born	:	8 – 26 mg/dL
Infants and Children	:	11 – 38mg/dL
Adults	:	15 – 39 mg/dL
Age >60 years	:	17 – 45 mg/dL [86]

10. SERUM CREATININE: (mg/dL)

Serum creatinine was determined by Jaffe's alkaline picrate method [87] in Micro 200 semi auto analyzer at 505nm.

PRINCIPLE:

Creatinine reacts with alkaline picrate and forms a red-orange adduct which is measured at 505 nm.

REFERENCE INTERVAL:

New Born	:	0.3 – 1.0 mg/dL
Infant	:	0.2 – 0.4 mg/dL
Children	:	0. 3 –0.7 mg/dL
Adolescent	:	0.5 – 1.0 mg/dL
Adult male	:	0.7 – 1.3 mg/dL
Adult Female	:	0.6 – 1.1 mg/dL [88]

RESULTS AND STATISTICAL ANALYSIS

The total number of subjects included for the study was 100. Out of 100, 50 were cases (Patients with Chronic kidney disease on hemodialysis) and 50 controls.

The distribution of age among the control group and the study group were as shown in Table No.21

The patients and the controls were grouped in to four according to the age as shown in Table No.22

The distribution of sex among the control group and the study group were as shown in Table No.23

Mean and standard deviation were estimated for each group.i.e cases and controls. Data were expressed as mean ± standard deviation.

Mean values were compared using student independent 't' test.

Student independent 't' test was employed to find out the 'p' value.

Age group wise comparisons of mean level of trace elements in study and control group were given in Table No. 25 to Table No.28.

Sex wise comparison of mean level of trace elements in study and control group was given in Table No.29 and Table No.30.

Pearson's correlation analysis was done to assure the relationship of Iron with Copper and Zinc in the study group and was given in Table No. 31. The results of the present study were given in Table No.24.

TABLE NO.21

AGE DISTRIBUTION AMONG THE STUDY AND CONTROL GROUP

Group	N	Mean Age (Years)	Standard Deviation	Student t-test
Study	50	41.36	15.053	P=0.82 Not significant
Control	50	40.68	13.969	

TABLE NO.22

GROUPING OF STUDY AND CONTROL MATERIALS BASED ON AGE

Age in Years	Group		Total	Chi square Test
	Study	Controls		
<25	9	9	18	$\chi 2=0.93$ P=0.81 Not significant
25-40	16	16	32	
40-60	17	20	37	
>60	8	5	13	
Total	50	50	100	

TABLE NO.23

DISTRIBUTION OF SEX AMONG THE STUDY GROUP AND CONTROL GROUP

Sex	Group		Total	Chi square Test
	Study	Control		
Male	33	33	66	$\chi 2=0.00$
Female	17	17	34	P=1.00
Total	50	50	100	Not significant

TABLE NO.24

MEAN, STANDARD DEVIATION AND TEST OF SIGNIFICANCE OF MEAN VALUES BETWEEN STUDY GROUP AND CONTROL GROUP

Variable	Study Group	Control Group	P Value
	Mean ± S.D	Mean ± S.D	
Blood Urea (mg/dL)	142.56 ± 28.871	23.92 ± 5.795	<0.001 Significant
Serum Creatinine (mg/dL)	7.97±3.184	0.98± 0.195	<0.001 Significant
Serum Iron (µg/dL)	90.59±32.821	103.38 ± 20.565	<0.02 Significant
Serum TIBC (µg/dL)	629.22±147.250	319.91± 50.081	<0.001 Significant
Serum Ferritin (ng/mL)	73.82±36.012	103.24± 39.997	<0.001 Significant
Serum Transferrin Saturation (%)	14.94±6.141	32.31± 4.047	<0.001 Significant
Serum Transferrin (mg/dL)	439.92±102.906	224.19± 35.575	<0.001 Significant
Serum copper (µg/dL)	77.65±37.438	103.22 ± 27.226	<0.001 Significant
Serum ceruloplasmin (Units)	234.46±80.952	332.14± 52.915	<0.001 Significant
Serum zinc (µg/dL)	57.42±17.869	101.48 ±18.076	<0.001 Significant

TABLE NO.25

AGE GROUPWISE COMPARISON OF MEAN LEVELS OF TRACE ELEMENTS IN STUDY SUBJECTS AND CONTROLS.

AGE < 25 YEARS

Variable	Cases	Controls	P Value
	Mean ± SD	Mean ± SD	
Blood Urea (mg/dL)	122.22±33.607	24.44±5.411	< 0.001 significant
Serum Creatinine (mg/dL)	6.57±2.515	0.96±0.142	< 0.001 significant
Serum Iron (µg/dL)	74.48±28.441	96.29±22.049	< 0.08 Not significant
Serum TIBC (µg/dL)	587.51±121.546	304.41±38.799	<0.001 significant
Serum Ferritin (ng/mL)	62.31± 21.298	107.89±53.629	<0.06 Not significant
Serum Transferrin Saturation (%)	12.77±4.195	31.59±5.579	< 0.001 significant
Serum Transferrin (mg/dL)	410.78±84.878	213.07±27.158	< 0.001 significant
Serum copper (µg/dL)	93.99±47.317	104.62±22.858	< 0.55 Not significant
Serum ceruloplasmin (Units)	260.00±112.299	365.56±65.740	< 0.05 significant
Serum zinc (µg/dL)	55.00±12.826	106.44±17.067	< 0.001 significant

TABLE NO.26

AGE 25 – 40 YEARS

Variable	Cases	Controls	P Value
	Mean±SD	**Mean ± SD**	
Blood Urea (mg/dL)	149.13±26.374	22.06±5.916	< 0.001 significant
Serum Creatinine (mg/dL)	8.22±3.204	0.94±0.190	< 0.001 significant
Serum Iron (µg/dL)	110.90±39.344	108.57±21.376	< 0.83 Not Significant
Serum TIBC (µg/dL)	624.05±197.356	323.32±47.083	< 0.001 significant
Serum Ferritin (ng/mL)	85.33±42.072	96.24±28.646	< 0.06 Not significant
Serum Transferrin Saturation (%)	19.05±8.062	33.50±4.215	< 0.001 significant
Serum Transferrin (mg/dL)	436.38±138.124	226.31±32.965	< 0.001 significant
Serum copper (µg/dL)	80.03±39.670	110.75±30.014	<0.01 significant
Serum ceruloplasmin (Units)	225.81±89.066	334.25±41.760	< 0.001 significant
Serum zinc (µg/dL)	56.88±19.609	102.31±17.484	< 0.001 significant

TABLE NO.27

AGE 41 – 60 YEARS

Variable	Cases	Controls	P Value
	Mean±SD	Mean ± SD	
Blood Urea (mg/dL)	148.76±26.459	24.85±6.293	< 0.001 significant
Serum Creatinine (mg/dL)	9.01±3.635	1.00±0.218	<0.001 significant
Serum Iron (µg/dL)	82.86±26.626	103.23 ± 20.507	< 0.01 significant
Serum TIBC (µg/dL)	618.67 ± 107.593	321.47 ± 57.491	< 0.001 significant
Serum Ferritin (ng/mL)	70.39 ±40.199	105.48 ± 46.798	<0.05 significant
Serum Transferrin Saturation (%)	13.57 ± 4.273	32.16 ±3.451	< 0.001 significant
Serum Transferrin (mg/dL)	432.71 ± 75.332	223.97± 41.359	< 0.001 significant
Serum copper (µg/dL)	64.35 ± 25.075	96.39 ± 28.039	<0.001 significant
Serum ceruloplasmin (Units)	222.35 ± 47.202	317.25 ±55.781	<0.001 significant
Serum zinc (µg/dL)	59.59 ± 17.418	96.00 ± 17.290	<0.001 significant

TABLE NO.28

AGE > 60 YEARS

Variable	Cases	Controls	P Value
	Mean±SD	Mean ± SD	
Blood Urea (mg/dL)	139.13 ± 26.755	25.20 ± 3.564	< 0.001 significant
Serum Creatinine (mg/dL)	6.83 ± 2.172	1.04±0 .230	< 0.001 significant
Serum Iron (µg/dL)	84.53 ± 14.654	100.12 ± 16.210	<0.10 Not significant
Serum TIBC (µg/dL)	708.88 ± 123.092	330.60 ± 54.395	< 0.001 significant
Serum Ferritin (ng/mL)	72.66 ± 29.238	112.92 ± 32.358	< 0.05 significant
Serum Transferrin Saturation (%)	12.03 ± 1.981	30.33±1.827	< 0.001 significant
Serum Transferrin (mg/dL)	495.13 ± 85.503	241.86 ± 34.707	< 0.001 significant
Serum copper (µg/dL)	83.50 ± 40.740	103.88 ± 21.406	< 0.34 Not significant
Serum ceruloplasmin (Units)	248.75 ± 88.144	324.80±25.114	< 0.09 Not significant
Serum zinc (µg/dL)	56.63 ± 22.570	111.80 ± 22.632	<0.001 significant

TABLE NO.29

SEX WISE COMPARISON OF MEAN LEVELS OF TRACE ELEMENTS IN STUDY SUBJECTS AND CONTROLS.
MALE

Variable	Cases	Controls	P Value
	Mean±SD	Mean ± SD	
Blood Urea (mg/dL)	143.76 ± 28.099	24.42 ± 5.668	< 0.001 significant
Serum Creatinine (mg/dL)	8.08 ± 3.380	0.98 ± 0.192	<0.001 significant
Serum Iron (µg/dL)	93.72 ± 31.598	102.41 ± 19.469	<0.18 Not significant
Serum TIBC (µg/dL)	625.79 ± 129.126	321.11 ±48.652	<0.001 significant
Serum Ferritin (ng/mL)	72.60 ± 33.042	109.58 ± 37.856	<0.001 significant
Serum Transferrin Saturation (%)	15.67 ± 6.602	31.93 ± 4.179	<0.001 significant
Serum Transferrin (mg/dL)	437.45 ± 90.106	225.20 ± 34.835	<0.001 significant
Serum copper (µg/dL)	80.96 ± 38.126	100.82 ± 24.889	<0.01 significant
Serum ceruloplasmin (Units)	239.15 ± 74.534	341.79 ± 49.939	<0.01 significant
Serum zinc (µg/dL)	57.94 ± 18.048	100.97 ± 17.758	<0.001 significant

TABLE NO.30

FEMALE

Variable	Cases	Controls	P Value
	Mean±SD	Mean ± SD	
Blood Urea (mg/dL)	140.24 ± 31.061	22.94 ±6.088	<0.001 significant
Serum Creatinine (mg/dL)	7.75 ± 2.850	0.96 ± 0.206	<0.001 significant
Serum Iron (µg/dL)	84.51 ±35.251	105.25 ± 23.053	<0.05 significant
Serum TIBC (µg/dL)	635.86 ±181.617	317.57 ±54.206	<0.001 significant
Serum Ferritin (ng/mL)	75.73± 41.295	93.74 ± 42.444	<0.23 Not significant
Serum Transferrin Saturation (%)	13.51 ± 5.002	33.04 ±3.789	<0.001 significant
Serum Transferrin (mg/dL)	444.71 ± 127.106	222.30 ± 37.945	< 0.001 significant
Serum copper (µg/dL)	70.81 ± 36.196	107.88 ± 31.562	< 0.01 significant
Serum ceruloplasmin (Units)	225.35 ± 93.940	313.41 ± 54.978	<0.01 significant
Serum zinc (µg/dL)	56.41 ± 18.021	102.47 ±19.193	<0.001 significant

TABLE NO.31

CORRELATION OF SERUM IRON WITH COPPER AND ZINC IN CHRONIC KIDNEY DISEASE PATIENTS UNDERGOING HEMODIALYSIS

Variables	Pearson's Correlation Coefficient (r)	Significance (p)	Interpretation
Iron Vs Copper	0.294	0.04	Significant and Positive Correlation
Iron Vs Zinc	0.18	0.28	Not significant and Positive correlation

Correlation always lies between –1 to +1

It is represented by 'r'.

DISCUSSION

Recent studies have focused on the possible role of trace element depletion in the pathogenesis of uremic symptoms such as anemia, dysguesia and impotence. It is well known that patients on long-term hemodialysis are at risk of developing changes in the trace element metabolism[89], which can further induce different abnormalities in these patients. Such abnormalities of trace element metabolism and their concentrations have been reported.

Lawson DH et al[90] has reported iron deficiency in patients with chronic kidney disease on regular hemodialysis treatment. In the present study, comparison of metabolic indicators of iron in patients with chronic kidney disease on hemodialysis with control group (Table No.24) reveals significant changes in the iron metabolism. This is evidenced by statistically significant decrease in serum iron, transferrin saturation and ferritin, and increase in serum transferrin and total iron binding capacity levels in the study group.

Comparison of indicators of iron metabolism between age matched study and control group and in both sexes (Table No.25 to Table No.30) did not show a uniform significance. This is probably because of the small number of patients taken for the study.

Iron deficiency is common in chronic kidney disease particularly in hemodialysis patients for several reasons:[91,92]

1. Substantial loss of blood from frequent blood tests and retention of blood in the dialysis tubing and the dialyser.

2. Blood loss in the gastrointestinal tract that cannot be compensated for by sufficient absorption from the diet.
3. Recombinant human erythropoietin administered to correct anemia in patients on hemodialysis induces a rise in hemoglobin concentration and depletes the body iron stores.

In the present study, comparison of the levels of serum copper, ceruloplasmin and zinc in study group with control group (Table No.24) reveal abnormalities in copper and zinc levels in patients on hemodialysis. It has been found that serum copper levels are significantly lower than control subjects, but are within normal reference range[83.] The levels of serum ceruloplasmin and zinc are significantly lower than the reference range[81, 84] in the study group suggesting their deficiency states. In this study, it has been found that, there is no significant influence of age and sex on copper and zinc levels (Table No.25 to Table No.30).

Yilmaz M. Emin et al[93] have reported significantly low plasma zinc and copper levels in patients on hemodialysis compared to normal subjects. Nancy J Emenaker et al[94] have reported low plasma copper and ceruloplasmin activities in patients undergoing hemodialysis with membranes that are not copper based. Another study by Van Renterghem D et al[95] have reported low serum zinc levels in patients on hemodialysis. This low level of zinc is explained by the removal of this element when the dialysate used contains sufficiently low concentration of zinc.

The deficiency of copper, ceruloplasmin and zinc in chronic kidney disease has been attributed to excessive loss in urine due to proteinuria. When patients on dialysis are given oral iron therapy (as ferrous sulphate), zinc absorption is reduced due to competition of divalent trace elements for the divalent metal transporter (DMT-1). This reduced absorption of zinc and the loss of zinc in dialysis fluid results in zinc depletion and deficiency in patients on frequent dialysis. As zinc is a cofactor for δ – amino levulinate dehydratase[96] in heme synthesis, zinc deficiency can interfere with hemoglobin synthesis and results in anemia. Zinc depletion in patients on hemodialysis leads to anorexia, dysguesia, hyposmia, dysosmia, impotence and impaired immunity. Zinc deficiency also correlates with signs of malnutrition in patients on hemodialysis[97].

Low serum copper and ceruloplasmin levels cause deficient ferroxidase activity and interfere with iron transport. This results in iron deficiency symptoms like anemia. The Karl Pearson Correlation Coefficient between serum iron and copper (Table No.31) is calculated and compared in the study group. A significant positive correlation between these two parameters is observed. Similarly, comparison of the Karl Pearson correlation coefficient between serum iron and zinc (Table No.31) shows a non-significant and positive correlation between these parameters. This shows the linear relationship between serum iron, copper and zinc in the study group.

Trace element supplementation to the patients with chronic kidney disease on long-term hemodialysis is necessary to avert the complications of trace element depletion. Patients on maintanence hemodialysis become iron depleted and require iron therapy. In a study on patients with hemodialysis associated anemia, untreated patients suffer a net iron deficit of approximately 1 mg/day.[98] The exact amount of iron needed and best route of administration are still uncertain. Carter et al[99] has suggested that oral iron therapy is inconsistent in its effects and parenteral iron should be given to patients. Another study by Eschbach et al[100] has found that absorption of inorganic iron is normal in chronic kidney disease and Brozovich et al[101] has suggested that oral iron is as effective as parenteral iron.

Zinc and copper deficiency occur together in patients on frequent dialysis and aggravate the iron deficiency anemia. Probably, providing supplements of zinc and copper can augment treatment of the anemia associated with frequent dialysis. Supplementation of iron, copper and zinc to the patients on hemodialysis should be adequately spaced to avoid interference in their absorption from gastrointestinal tract. Trace mineral supplementation will probably improve the quality of life in patients with kidney failure on hemodialysis.

CONCLUSION

The present study shows that there is a disturbance in the metabolism of trace elements such as iron, copper and zinc in patients with chronic kidney disease on long-term hemodialysis. Trace element supplementation is necessary to improve the morbidity and clinical status in patients undergoing hemodialysis.

SCOPE FOR FURTHER STUDY

- Further studies are needed to elucidate the clinical impact of other trace elements such as Aluminium, Magnesium, Molybdenum, Cadmium, Lead, Selenium and Cobalt on patients undergoing long-term hemodialysis.

- Newer methods of measuring iron status in patients on hemodialysis such as reticulocyte hemoglobin content and serum transferrin receptor and percent hypochromic red blood cells can be used that are more sensitive and specific than current iron parameters.

- Trace element concentration in the dialysate fluid can be estimated to evaluate the possible consequences of contamination with the trace elements e.g. Aluminium.

TABLE NO.1

STAGES OF CHRONIC KIDNEY DISEASE[13]

Stage	Description	GFR mL/min/1.73m^2
	At Increased risk	90 (With Chronic Renal Disease risk factors)
1	Kidney damage with normal or increased GFR	90
2	Kidney damage with mildly decreased GFR	60-89
3	Moderately decreased GFR	30-59
4	Severely decreased GFR	15-29
5	Renal Failure	<15 (or dialysis)

TABLE NO. 2

FOODS RICH IN HEME AND NON-HEME IRON

Heme iron	Non heme iron
Liver Meat Poultry Fish	Green leafy vegetables Cereals Nuts Legumes Oilseeds Jaggery Dried fruits

TABLE NO.2 (a)

RECOMMENDED DAILY INTAKE OF IRON [28(a)]

Category	Age	Recommended daily intake (mg)
Infants (Full term)	0 – 3 months 3- 6 months 6- 12 months	- 6.6 8.8
Children (Male and Female)	1 – 10 years	10
Male	10 – 18 years >18 years	12 10
Females Non-Pregnant Pregnant Postmenopausal	10- 45 years	15 45 10

TABLE NO.3

COMPARTMENTS OF IRON AND ITS COMPOSITION:
(Average 70 kg man) [32]

Compartment	**Iron Content (mg)**	**Total Body Iron (%)**
Hemoglobin	2000	67
Storage Iron (Ferritin, Hemosiderin)	1000	27
Myoglobin	130	3.5
Labile Pool	80	2.2
Transport Iron	3	0.08
Other Tissue Iron	8	0.2

TABLE NO. 4

CLINICAL SIGNIFICANCE OF SERUM IRON

Decreased	Increased
Physiological	**Physiological**
Diurnal- Evening Menstruation	Morning Oral Contraceptives
Pathological	**Pathological**
Iron deficiency Chronic Disease- Renal Failure Malignancy Inflammation Recent Blood Loss	Ineffective Erythropoiesis Megaloblastic anemia Thalassemia major Sideroblastic anemia Hemolytic anemia Aplastic anemia Viral hepatitis Hemochromatosis Acute iron poisoning

TABLE NO.5

CLINICAL SIGNIFICANCE OF SERUM TRANSFERRIN AND TIBC

Decreased	Increased
Physiological	**Physiological**
	Pregnancy Oral Contraceptives
Pathological	**Pathological**
Inflammation Infection Malignancy Liver disease Nephrotic syndrome Malnutrition Megaloblastic and Hemolytic anemia Hemochromatosis	Iron Deficiency Hepatitis

TABLE NO.6

CLINICAL SIGNIFICANCE OF SERUM TRANSFERRIN SATURATION

Decreased	Increased
Physiological	**Physiological**
Diurnal- Evening Pregnancy	Morning
Pathological	**Pathological**
Chronic Disease	Hemochromatosis Thalassemia Major Sideroblastic anemia Acute iron poisoning

TABLE NO.7

CHANGES IN SERUM FERRITIN

Decreased	Increased
Iron deficiency anemia	Fever
Hypothyroidism	Acute infection
Ascorbate deficiency	Rheumatoid arthritis
	Chronic inflammatory disease
	Liver damage (acute or chronic)
	Iron overload (Hemochromatosis)

TABLE NO.8

RECOMMENED DAILY ALLOWANCE FOR ZINC[43]

	Age(Years)	**Males (mg/dL)**	**Females (mg/dL)**
Infants	0 - 0.5	5	5
	0.5 – 1	5	5
Children	1 – 3	10	10
	4 – 6	10	10
	7 – 10	10	10
Adolescents	11 – 14	15	12
	15 – 18	15	12
Adults	19 – 24	15	12
	25- 50	15	12
	> 50	15	12
Pregnancy			15
Lactation	0 – 6 Months		19
	7 – 12 Months		16

TABLE NO. 9

PLASMA ZINC LEVELS IN NORMAL SUBJECTS AND PATIENTS WITH RENAL FAILURE[59]

Group	Plasma Zinc Levels(μg/dL)
Normal Subjects	81± 13
	84 ± 11
	108 ± 20
	96 ±13
	85 ±18
	50 ± 9
Hemodialysis patients	63 ±5
	71 ± 7
	261 ± 92
	80 ± 16
	102 ± 32
Uremic patients not treated with dialysis	117 ± 15
	65 ± 13
	81 ±11

TABLE NO. 10

PREPARATION OF WORKING STANDARDS FOR IRON:

Working Standard Concentration (μg/dL)	**Stock Solution mL**	**Deionised water mL**
50	0.05	9.95
100	0.10	9.90
200	0.20	9.80
300	0.30	9.70
400	0.40	9.60

TABLE NO.14

REFERENCE INTERVAL FOR SERUM TRANSFERRIN

Serum reference intervals based on CRM 470[79] are as follows.

Age	g/L	mg/dL
Newborn	1.17 – 2.50	117 – 250
Adults (20 – 60 yr)	2.0 – 3.6	200 – 360
> 60 yr	1.6 – 3.4	160 – 340

TABLE NO. 15

PREPARATION OF WORKING STANDARDS FOR ZINC

Working Standard (µg / dL)	**Available Zinc Concentration (200µg/dL) (µL)**	**Deionised Water (µL)**
50	25	75
100	25	25

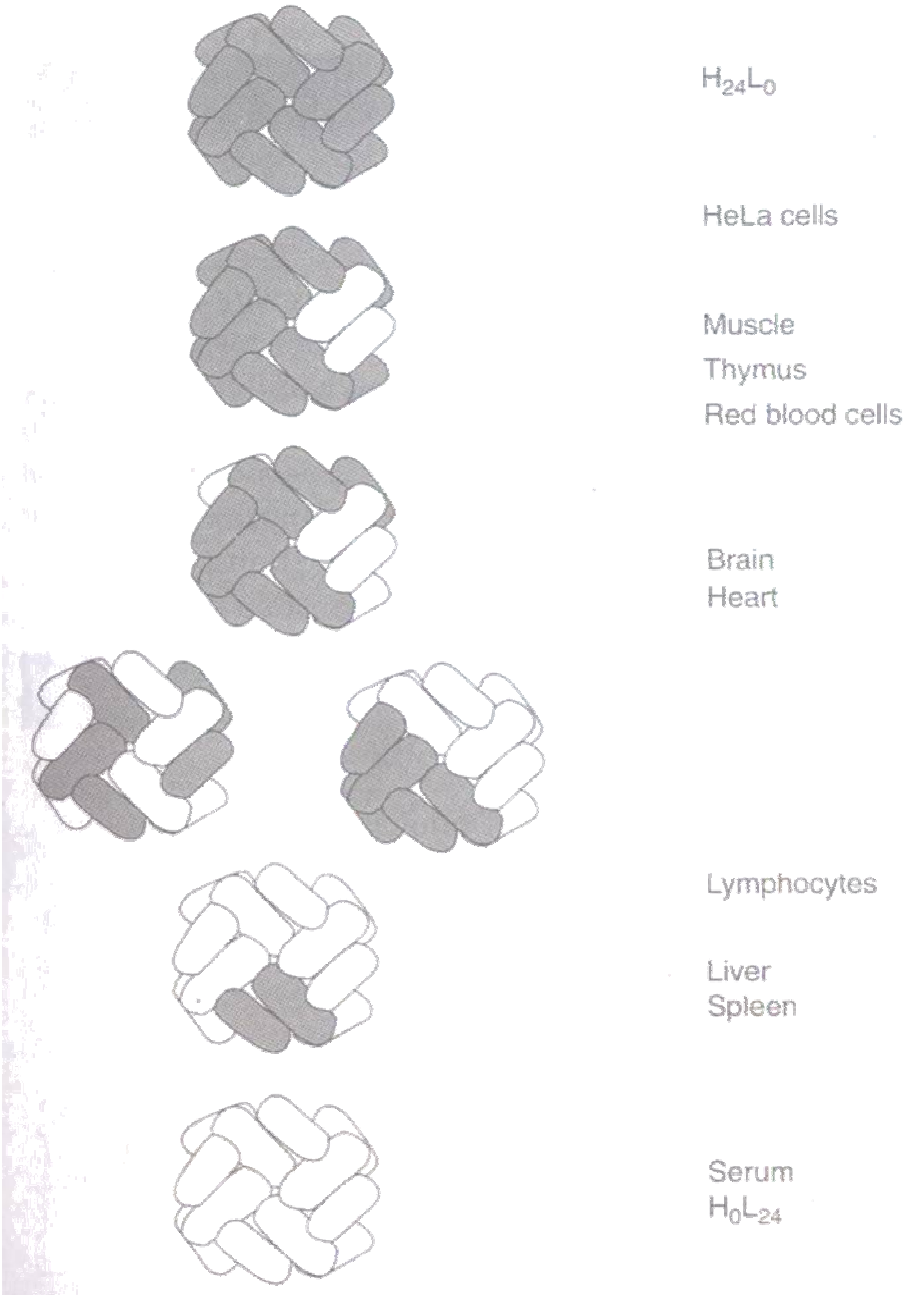
TABLE NO. 18

PREPARATION OF WORKING STANDARDS FOR COPPER

Working Standard Concentration (μg/dL)	**Stock Solution (mL)**	**Deionised Water (mL)**
50	0.5	99.5
100	1	99
200	2	98
300	3	97
400	4	96

FIGURE NO. 4

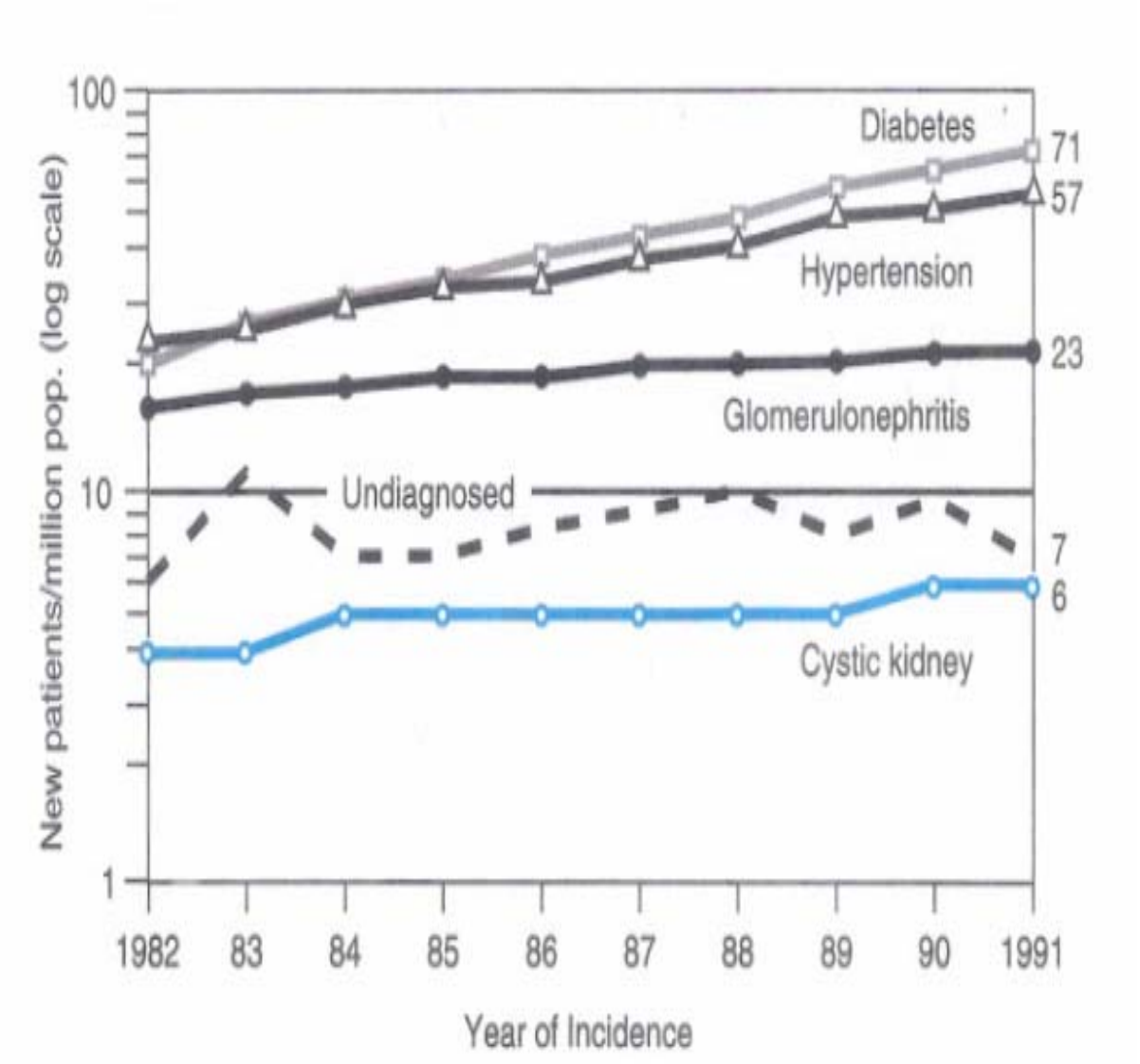
SCHEMATIC REPRESENTATION OF THE SUBUNIT STRUCTURE OF FERRITIN



Reproduced from Harrison PM, Arosio P. The Ferritin: Molecular properties, iron storage function and cellular regulation, Biochim Biophys Acta 1996;1275:161- 203

FIGURE NO.1

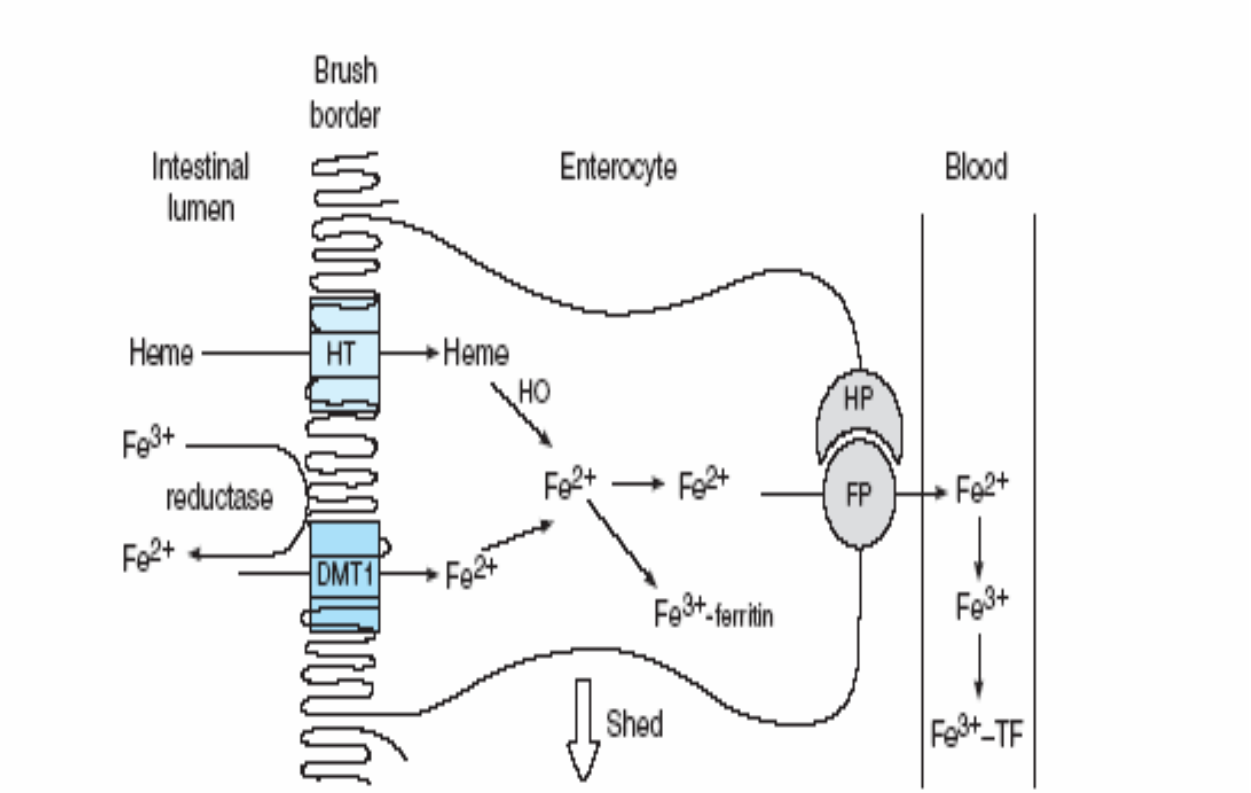
INCIDENCE RATES OF TREATED END-STAGE RENAL DISEASE (ESRD) PER MILLION POPULATION FROM 1982 TO 1991



Reproduced from United States Renal Data System, 1995. Annual Data Report, U.S. Department of Health and Human Services, Health Care Financing Administration.

FIGURE NO.3

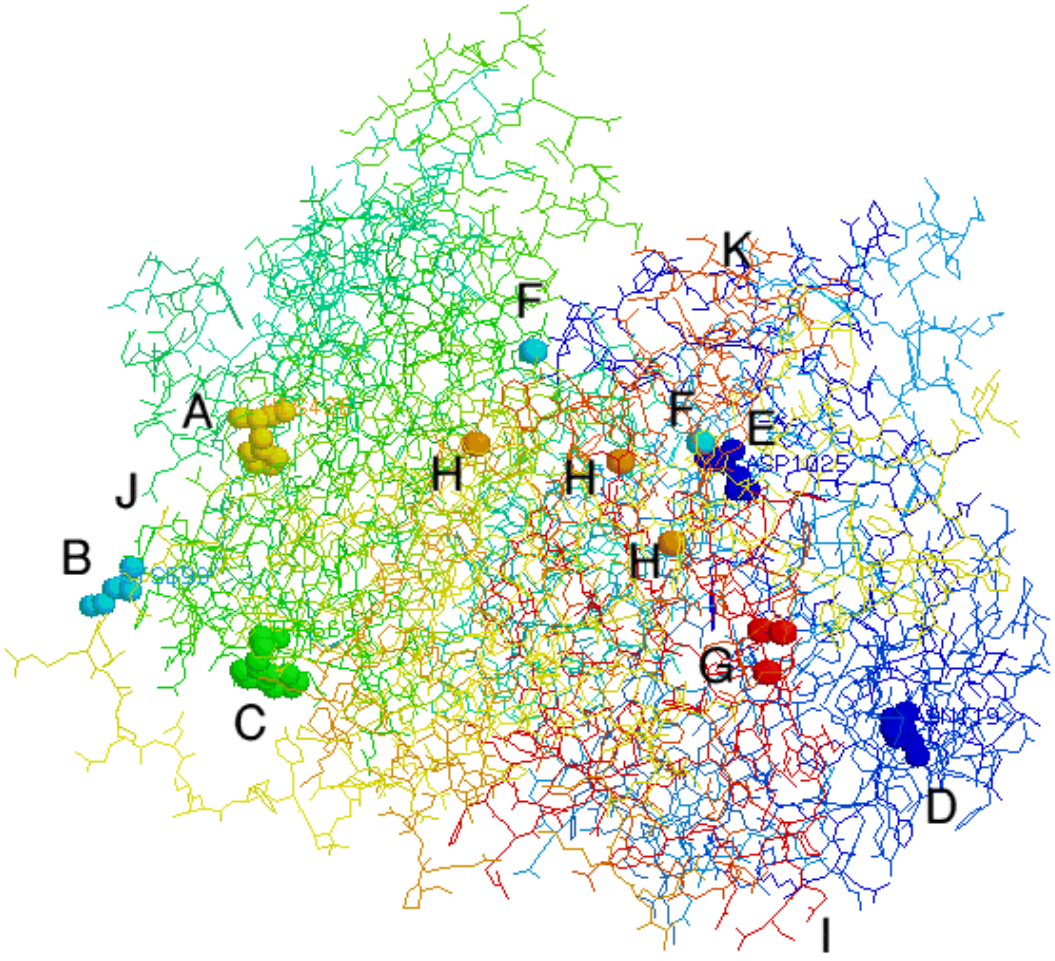
ABSORPTION OF IRON IN THE GASTROINTESTINAL TRACT



Reproduced from Ganong WF: Review of Medical Physiology, 2003, 21st edition, Page No.482. McGraw-Hill.

FIGURE NO. 5

STRUCTURE AND FUNCTIONAL SITES IN HUMAN CERULOPLASMIN



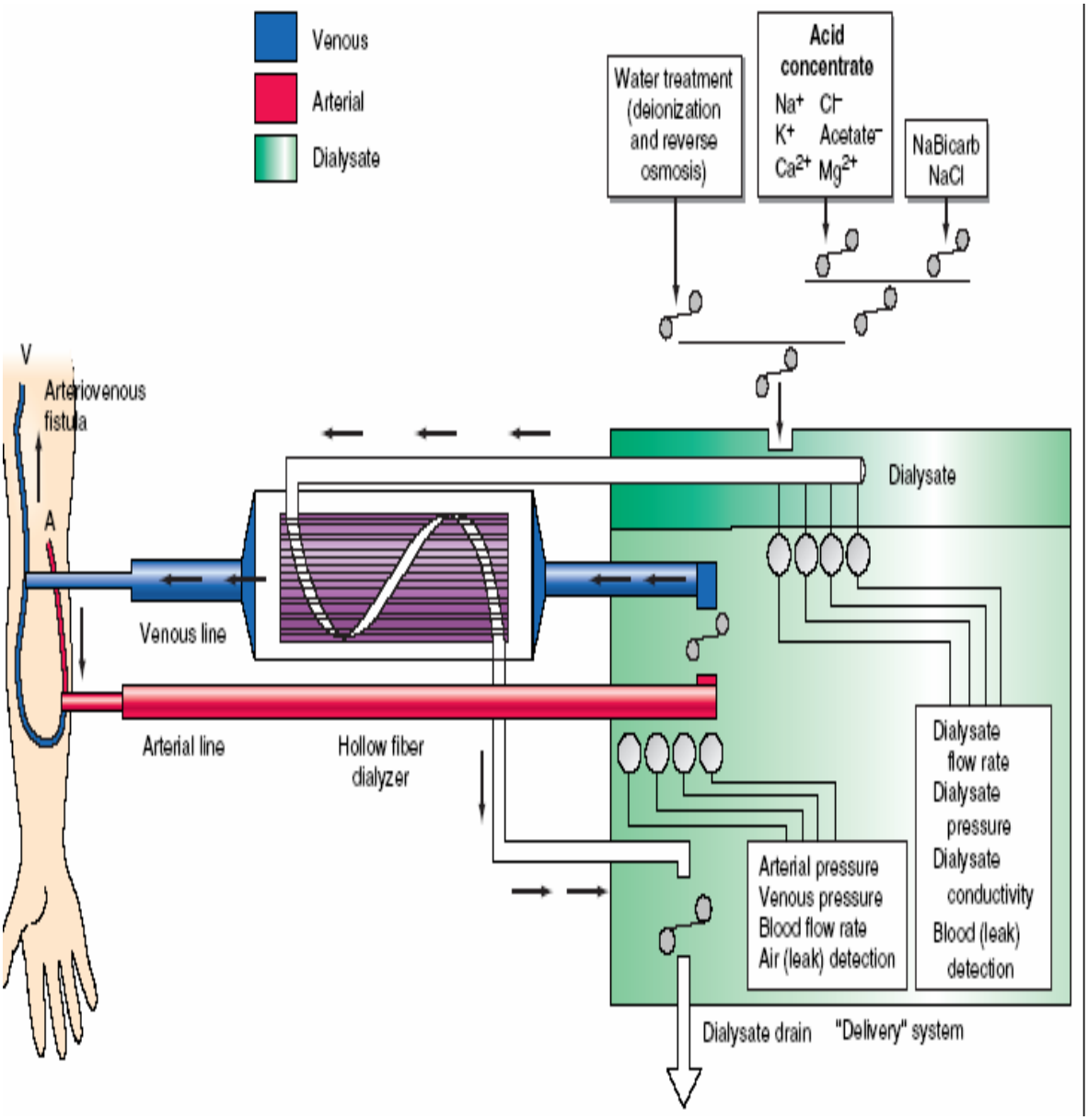
Binding and Active Sites

Pro-oxidant: A	Copper: F, G, H
Peroxidase: B	NO: I,J
Amine: C	Fe^{3+}: K
LSD: D	Fe^{2+I}: E

REPRODUCED FROM WEBSITE**:** E:\ caeruloplasmin structure – function pdb file display. htm.

FIGURE NO. 2

SCHEMA FOR HEMODIALYSIS



Reproduced from Charles D. Carpenter and J. Michael Lazarus. Dialysis and Transplantation in the treatment of renal failure. Harrison's Principle and Practice of Internal Medicine 1998, 14th edition Volume II, Page No. 1522. Mc Graw – Hill.

BIBLIOGRAPHY

1. Karl Skorecki, Jacob Green, and Barry M. Brenner. Chronic Renal Failure. **Harrison's Principles and Practice of Internal Medicine** 2005, 16th edition, edited by Dennis L.Kasper, Eugene Braunwald, Anthony S. Fauci, Stephen L. Hauser, Dan L. Longo, J. Larry Jameson, volume II, Page No.1653. McGraw – Hill Medical Publishing Division.

2. Suresh Chandra Dash and Sanjay K Agarwal. Incidence of Chronic kidney Disease in India, **Nephrology Dialysis Transplantation** 2006; 21(1): 232 – 233.

3. Raj B and Jayaraman M A. Global Perspective, **Dialysis Transplantation** 1991; 20: 470.

4. Rashad S. Barsom, Visith Sitprija. Tropical Nephrology. **Diseases of the Kidney** 1997, 6th edition, edited by Robert W.Schrier, Carl W. Gottschalk, volume III, Page No.2251 – 2252. Little Brown and Company.

5. National Kidney Foundation. K/DOQI: Clinical Practice Guidelines for Chronic Kidney Disease. Evaluation, Classification, and Stratification. Kidney Disease Outcome Quality Initiative, **American Journal of Kidney Disease** 2002; 39: S1 – 246.

6. Biff F.Palmer. Dialysate composition in Hemodialysis and Peritoneal Dialysis. **Principles and Practice of Dialysis** 1994, edited by William L. Henrich, page No.8. Williams and Wilkins.

7. Ruggenenti P, Schieppati A, Remuzzi G. Progression, remission of Chronic renal diseases, **Lancet** 2001; 357:1601 – 1608.

8. Lysaght M J. Maintanence dialysis population dynamics: Current trends and long term implications, **Journal of American Society of Nephrology** 2002; 13: S37 – S40.

9. **US Renal Data System**, USRDS 2005. Annual Data Report: Atlas of End-Stage Renal Disease in the United States. Bethesda, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 2005.

10. Mani M. Chronic renal Failure in India, **Nephrology Dialysis Transplantation** 1993; 8: 684.

11. J.Michael Lazarus, Barry M.Brenner. Chronic Renal Failure. **Harrison's Principles and Practice of Internal Medicine** 1998, 14th edition, edited by Dennis L.Kasper, Eugene Braunwald, Anthony S. Fauci, Stephen L. Hauser, Dan L. Longo, J. Larry Jameson, volume II, Page No.1513. McGraw – Hill Medical Publishing Division.

12. Lee-Ellen C Copstead, Jacquelyn L Banasik. Renal failure. **Pathophysiology** 2005, 3rd edition, Page No. 743. Elsevier Saunders.

13. Levey A S et al. National Kidney Foundation K/DOQI Clinical Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification and Stratification, **American Journal of Kidney Disease** 2002; 39(Suppl 1): S1.

14. Vanholder R C, Ringoir S M. Adequacy of dialysis: a critical analysis, **Kidney International** 1992; 42: 540 – 558**.**

15. Karl Skorecki, Jacob Green, and Barry M.Brenner. Chronic Renal Failure**. Harrison's Principles and Practice of Internal Medicine**,

2005, 16th edition, edited by Dennis L.Kasper, Eugene Braunwald, Anthony S.Fauci, Stephen L.Hauser, Dan L.Longo, J. Larry Jameson. Volume.II, Page No.1654 – 1655. McGraw – Hill Medical Publishing Division.

16. Michael P.Delaney, Christoper P.Price, David J.Newman, Edmund Lamb. Kidney Disease**. Tietz textbook of clinical chemistry and molecular diagnostics** 2006, 4th edition, edited by Carl A Burtis, Edward R Ashwood, David E Bruns. Page No. 1718 – 1719. Elsevier Saunders.

17. William Drukker. Hemodialysis: a historical review. **Replacement of renal function by dialysis** 1983, 2nd edition, edited by William Drukker, Frank M. Parsons and John F. Maher. Page No. 7 – 13. Martinus Nijhoff Publishers.

18. Charles D. Carpenter, J.Michael Lazarus. Dialysis and Transplantation in the treatment of Renal Failure. **Harrison's Principles and Practice of Internal Medicine** 1998, 14th edition, edited by Dennis L.Kasper, Eugene Braunwald, Anthony S. Fauci, Stephen L. Hauser, Dan L. Longo, J. Larry Jameson, volume II, Page No.1522. McGraw – Hill Medical Publishing Division.

19. Christoph Olbricht, Gerhard Lonnomann, Ulrich Frei, and Karl – Martin Koch. The Dialysis Patient: Hemodialysis, Hemofiltration, and Complications of technique**. Oxford Text Book of Clinical Nephrology** 1998, 2nd edition, Vol.III, edited by Alex M. Davison, Stewart Cameron, Jean – Pierre Grunfeld, David N.S.Kerr, Eberhard Ritz and Christoper G. Winearls. Page No.2025. Oxford University Press.

20. Foley R N, Parfrey P S, Harnett J D, Kent G M, Martin C J, Murray D C et al. Clinical and echocardiographic disease in patients starting end stage renal disease therapy, **Kidney International** 1995; 47: 186 – 192.

21. Silberberg J S, Barre P E, Prichard S S, Sniderman A D. Impact of Left Ventricular Hypertrophy on survival in End Stage Renal Disease, **Kidney International** 1989; 36: 286 – 290**.**

22. Muirhead N, Kertesz A, Flanagan P R, Hodsman A B, Hollomby D J, Val berg L S. Zinc metabolism in patients on maintenance hemodialysis, **American Journal of Nephrology** 1986; 6(6): 422 – 426.

23. Hosokawa S, Nishitani H, Tomita K, Tomoyoshi T, Nishio T, Sawanishi K, Yoshida O. Serum copper concentration changes in chronic hemodialyzed patients, **Uremia Investigation** 1985-1986; 9(1): 63-67.

24. Hung K Y, Ho C Y, Kuo Y M, Lee S H, Hseih S J, Yang C S, Peng C J, Wu D J, Hung J T, Chen P Y, Chen J S, Chen W Y. Trace element burden in geriatric hemodialysis patients: a prospective multicenter collaborative study, **International Journal of Artificial Organs** 1997 Oct; 20(10): 553-556.

25. Alan Shenkin, Malcolm Baines, Gordon S.Fell, and T.D.G. Lyon. Vitamins and Trace elements. **Tietz textbook of clinical chemistry and molecular diagnostics** 2006, 4th edition, edited by Carl A Burtis, Edward R Ashwood, David E. Bruns. Page No.1118. Elsevier Saunders.

26. Kenneth R. Bridges and Paul A. Seligman. Disorders of iron metabolism. **Blood: Principles and practice of hematology** 1995, edited by Robert I. Handin, Samuel E. Lux and Thomas P. Stossel. Page No. 1433. J.B. Lippincott Company .

27. Finch CA, Huebers H. Perspectives in Iron metabolism, **New England Journal of Medicine** 1982; 306: 1520.

28. Finch CA, Huebers HA.Iron Metabolism, **Clinical Physiology and Biochemistry** 1986; 4:5.

28(a). Virgil F. Fairbanks. Iron in medicine and nutrition. **Modern nutrition in health and disease** 1999, 9th edition, edited by Maurice E. Shils, James A. Olson, Moshe Shike and A. Catharine Ross. Page No.209. Williams and Wilkins.

29. Green R et al. Body iron excretion in man, **American Journal of Nutrition** 1968; 45: 336.

30. Ganong WF. Digestion and Absorption. **Review of Medical Physiology** 2003, 21st edition, Page No.482. McGraw-Hill Publishing division.

31. Regulation of Iron Balance. Hepcidin.pp.ppt. Microsoft Power-Point Presentation**. www.acbd.monash.org**/regulation - of - iron – balance – sant – ryan – ppt.

32. Fairbanks V F, Beutler E. Iron Metabolism. In: Beutler E, Lichtman M A, Coller B S, Kipps T J, Seligsohn U, eds. **Williams Hematology**. New York. 2001. edited by Marshall A Lichtman, Thomas J Kipps, Kenneth Kaushansky, Ernest Beutler, Uriselighson, Josef T Prchal. Page No. 295 – 304. McGraw – Hill Publishing division**.**

33. Prem Ponka et al. Functions and Regulations of Transferrin and Ferritin, **Seminars in Hematology** 1998 January**;** 35: No.1: 35 – 54.

34. Harrison P M, Arosio P. The ferritin: Molecular properties, Iron storage function and cellular regulation, **Biochimca et Biophysica Acta** 1996; 1275:161 – 203.

35. Jerome L. Sullivan et al. The paradigm of Ischemic Heart Disease, **American Heart Journal** 1969; 117(5): 1177 – 1188.

36. A. Myron Johnson MD. Aminoacids, Peptides, and Proteins**. Tietz textbook of clinical chemistry and molecular diagnostics** 2006, 4th edition, edited by Carl A Burtis, Edward R Ashwood, David E. Bruns, Page No.563. Elsevier Saunders.

37. Trefor Higgins, Ernest Beutler MD and Basil T.Doumas Ph.D. Hemoglobin, Iron and Bilirubin. **Tietz textbook of clinical chemistry and molecular diagnostics** 2006, 4th edition; edited by Carl A Burtis, Edward R Ashwood, David E. Bruns, Page No1189. Elsevier Saunders.

38. A Jacob, F Miller et al. Ferritin in the serum of normal subjects and patients with Iron deficiency and Iron overload, **British Medical Journal** 1972; 28: 206 – 208.

39. Kerstin Klipstein – Grobusch et al. Serum Ferritin and risk of myocardial infarction in the elderly, **American Journal of Clinical Nutrition** 1999; 69: 1231 – 1236.

40. David M Baer. Iron stores are not associated with Acute Myocardial Infarction, **Circulation** 1994; 89: 2915 – 2918.

41. Christoper T. Sempsoz Ph.D et al. Body Iron stores and the risk of Coronary artery disease, **New England Journal of Medicine** 1994; 1119 – 1126.

42. National Kidney Foundation. K/DOQI: Guidelines for Anemia of Chronic Kidney Disease: Iron Support, **American Journal of Kidney Diseases** 1997 October; 30 (4), Suppl 3: S202 – 212.

43. Janet C. King and Carl L. Keen. Zinc. **Modern nutrition in health and disease** 1999, 9th edition, edited by Maurice E. Shils, James A. Olson, A. Catharine Ross, Page No. 234. Williams and Wilkins.

44. Lowe N M, Woodhouse L R, Matel J S, King J C. Comparison of estimates of zinc absorption in humans by using 4 stable isotopic tracer methods and compartmental analysis, **American Journal of Clinical Nutrition** 2000; 71: 523 – 529.

45. Chesters J K. Zinc. **Handbook of Nutritionally Essential Mineral Elements**. NewYork, 1997, edited by O'Dell B L, Sunde R A, Page No. 185 – 230. Marcel Dekker.

46. O'Brien K O, Zavaleta N, Caulfield L E, Wen J, Abranes S A. Prenatal iron supplements impair zinc absorption in pregnant Peruvian women, **Journal of Nutrition** 2000; 130: 2251 –2255.

47. Foote J W, Delves H T. Albumin bound and alpha 2 – macroglobulin bound zinc concentrations in the sera of healthy adults, **Journal of Clinical Pathology** 1984; 37:1050 – 1054.

48. King J C, Keen C L. Zinc. **Modern nutrition in health and disease**. Baltimore 1999. 9th edition, edited by Shils M E, Olson J A, Shihe M, Ross A C, Page No.223 –239. Williams and Wilkins.

49. Elia M, Crozier C, Neale G. Mineral metabolism during short – term starvation in man, **Clinica Chimica Acta** 1984; 139: 37 – 45.

50. Auld D S. Zinc coordination sphere in biochemical zinc sites, **Biometals** 2001; 14: 271–313.

51. Rink L. & Gabriel P. Zinc and the immune system, **Proceedings of the Nutrition Society** 2001; 59: 541–552.

52. Alan Shenkin, Malcolm Baines, Gordon S. Fell and T.D.G.Lyon. Vitamins and trace elements. **Tietz textbook of clinical chemistry and molecular diagnostics** 2006, 4th edition, edited by Carl A Burtis, Edward R Ashwood, David E. Bruns, Page No1138. Elsevier Saunders.

53. Falchuk KH. The molecular basis for the role of zinc in developmental biology, **Molecular and Cellular Biochemistry** 1998; 188: 41-48.

54. Urnov F D. A feel for the template: Zinc finger protein transcription factors and chromatin, **Biochemistry and Cell Biology** 2002; 80: 321-333.

55. Kenneth H Falchuk. Disturbances in Trace elements, **Harrison's Principles of Internal medicine** 1998, 14th edition, vol-I, edited by Dennis L.Kasper, Eugene Braunwald, Anthony S Fauci, Stephen L Hauser, Dan L Longo, J. Larry Jameson, Page No.491. Mc Graw – Hill Medical Publishing Division

56. Alan Shenkin, Malcolm Baines, Gordon S. Fell and T.D.G.Lyon. Vitamins and trace elements. **Tietz textbook of clinical chemistry and molecular diagnostics** 2006, 4th edition, edited by Carl A Burtis, Edward R Ashwood, David E. Bruns, Page No.1141. Elsevier Saunders.

57. Hambidge M. Human Zinc deficiency, **Journal of Nutrition** 2000; 130: 1344S - 9S

58. Prasad A. Effects of Zinc deficiency on immune functions, **Journal of Trace Elements in Experimental Medicine** 2000; 13: 1 – 20.

59. Geoffrey M. Berlyne. Uremia and the effects of Dialysis. Trace Metal Burden in Uremia, **Massry and Glassock's Textbook of Nephrology** 1995, 3rd edition, edited by Shaul G.Massry, Richard J.Glassock. Vol.II, Page No.1492 - 1493. Williams and Wilkins.

60. Andrew Sass – Kort sak, Copper Metabolism, **Advances in Clinical Chemistry** 1965, edited by Harry Sobotka, C.P. Stewart. Page No.1, Academic Press, New York and London.

61. Judith R. Turnlund. Copper. **Modern nutrition in health and disease**. Baltimore 1999. 9th edition, edited by Shils M E, Olson J A, Shihe M, Ross A C, Page No.223 –239, Williams and Wilkins.

62. Harris E D. Copper. **Handbook of nutritionally essential mineral elements** New York, 1997, edited by O' Dell BL, Sunde RA, Page No. 231 –273. Marcel Dekker.

63. Alan Shenkin, Malcolm Baines, Gordon S. Fell and T.D.G.Lyon. Vitamins and trace elements. **Tietz textbook of clinical chemistry and molecular diagnostics** 2006, 4th edition, edited by Carl A Burtis, Edward R Ashwood, David E. Bruns, Page No.1126 - 30. Elsevier Saunders.

64. Cordano A. Clinical manifestations of Nutritional Copper deficiency in infants and children, **American Journal of Clinical Nutrition** 1998; 687: 1012 S- 6 S.

65. Lonnerdal B. Copper Nutrition during Infancy and childhood, **American Journal of Clinical Nutrition** 1998; 67: 1046S – 53S.

66. Camblor M, De La Cuerda, Breton I et al. Copper deficiency with pancytopenia due to enteral nutrition through Jejunostomy, **Clinical Nutrition** 1997; 16: 129-31.

67. Klevay L M. Trace element and mineral nutrition in Ischemic heart disease. **Clinical nutrition of the essential trace elements and minerals**. Totowa, NewJersy 2000, edited by Bogden J D, Klevay L M, Page No.251 –271. Humana Press.

68. Ferenci P, Caca K, Loudianos G, Mieli–Vergani G, Tanner S, Sternlieb I et al. Diagnosis and phenotypic classification of Wilson disease, **Liver** 2003; 23: 139-142.

69. Gaffney D, Fell G S, O'Reilly D S. ACP Best practice No.163. Wilson's disease: Acute and presymptomatic laboratory diagnosis and monitoring, **Journal of Clinical Pathology** 2000; 53: 807-812**.**

70. Gitlin J D. Transcriptional regulation of ceruloplasmin gene expression during inflammation, **Journal of Biological Chemistry** 1988; 263:6281-7.

71. A. Myron Johnson, MD. Aminoacids, Peptides and Proteins, **Tietz text book of clinical chemistry and molecular diagnostics**, 2006, 4th edition, edited by Carl A Burtis, Edward R Ashwood, David E. Bruns, Page No.556. Elsevier Saunders.

72. A. Myron Johnson, MD. Aminoacids, Peptides and Proteins, **Tietz text book of clinical chemistry and molecular diagnostics** 2006, 4th edition, edited by Carl A Burtis, Edward R Ashwood, David E. Bruns, Page 557-558. Elsevier Saunders.

73. White J M and Flashka H A. An automated procedure with use of Ferrozine for assay of serum iron and total iron binding capacity, **Clinical Chemistry** 1973; 19: 526 – 528

74. Norbert W Tietz. Blood gases and Electrolytes, **Fundamentals of Clinical Chemistry** 1982, Page No. 929, W.B.Saunders Company.

75. Iron, Copper and Zinc. **Practical Clinical Biochemistry** 1980, 5th edition, Volume I, edited by Harold Varley, Alan H. Gowenlock and Maurice Bell, Page No.935 – 936. William Heinemann Medical Books Ltd.

76. Pennell C Painter, June Y Cope and Jane L Smith. Appendix. **Tietz Textbook of Clinical Chemistry** 1994, 2nd edition, edited by Carl A. Burtis and Edward R. Ashwood, Page No. 2188. W. B. Saunders Company.

77. Gambino R, Desvarieux E, Orth M, Matan H, Ackattupathil T, Lijoi E, et al. The relationship between chemically measured total iron-binding capacity concentrations and immunologically measured transferrin concentrations in human serum. **Clinical Chemistry** 1997; 43: 2408-2412

78. Vernet M. Immunochemical assay of transferrin and iron saturation in serum. **Clinical Chemistry** 1993; 39: 2352-2353

79. Dati F, Schumann G, Thomas L, Aguzzi F, Baudner S, Bienvenu J, et al. Consensus of a group of Professional societies and Diagnostic Companies on guidelines for interim reference ranges for 14 proteins in serum based on the standardization against the IFCC/BCR/CAP reference material (CRM 470), **European Journal of Clinical Chemistry and Clinical Biochemistry** 1996; 34: 517 – 520.

80. Pennell C Painter, June Y Cope and Jane L Smith. Appendix. **Tietz Textbook of Clinical Chemistry,** 1994, 2nd edition, edited by Carl A. Burtis and Edward R. Ashwood, Page No.2195. W. B. Saunders Company.

81. Pennell C.Painter, June Y Cope and Jane L Smith. Appendix. **Tietz Textbook of Clinical Chemistry** 1994, 2nd edition, edited by Carl A. Burtis and Edward R. Ashwood, Page No.2211. W. B. Saunders Company.

82. Iron, Copper and Zinc. **Practical Clinical Biochemistry** 1980. 5th edition Volume I, edited by Harold Varley, Alan H. Gowenlock and Maurice Bell. Page No.941 – 942. William Heinemann Medical Books Ltd.

83. Pennell C Painter, June Y Cope and Jane L Smith. Appendix**. Tietz Textbook of Clinical Chemistry** 1994, 2nd edition, edited by Carl A. Burtis and Edward R. Ashwood., Page No.2183. W. B. Saunders Company.

84. Henry R.J. **Clinical Chemistry, principles and Technics**. Newyork, Hoeber Med. Div., Harper and Row, 1964.

85. Sampson E J, Baird M A, Burtis C A, Smith E M, Witte D L, Bayse D D. A coupled – enzyme equilibrium method for measuring urea in serum: Optimisation and evaluation of the AACC Study Group on urea candidate reference method, **Clinical Chemistry** 1980; 26: 816 – 826.

86. Pennell C Painter, June Y Cope and Jane L Smith. Appendix. **Tietz Textbook of Clinical Chemistry** 1994, 2nd edition, edited by Carl A. Burtis and Edward R. Ashwood, Page No.2209. W. B. Saunders Company.

87. Larsen K. Creatinine assay by a reaction – kinetic principle, **Clinica Chimica Acta** 1972; 41: 209.

88. Pennell C Painter, June Y Cope and Jane L Smith. Appendix. **Tietz Textbook of Clinical Chemistry** 1994, 2nd edition, edited by Carl A. Burtis and Edward R. Ashwood, Page No.2184. W. B. Saunders Company.

89. Krachler M, H Scharfetter, G H Wirnsberger, **Clinical Nephrology** 2000; 54: 35.

90. Lawson D H et al. Iron Metabolism in patients with Chronic Renal Failure on regular hemodialysis treatment, **Clinical Science** 1971; 4: 345**.**

91. Van wyck DB. Iron deficiency in patients with dialysis associated anemia during erythropoietin replacement therapy. Strategies for assessment and management, **Seminars in Nephrology** 1995; 9: (Suppl 2): 21 – 24.

92. Kooistra M P, Van E S A, Struyvenberg A, Marx J J M. Low iron absorption in erythropoietin treated hemodialysis patients, **Journal of American Society of Nephrology** 1995; 6: 543A.

93. Yilmaz M.Emin, Kiraz Mehmet, Ismail Hamdi Kara. The evaluation of serum zinc and copper levels in hemodialysis patients in Southeast Turkey, **Dialysis Transplantation** 2000; 29: 718 – 746.

94. Nancy J Eminaker, Robert A Disilvestro, N Stanley Nahman Jr and Susan Percival. Copper related blood indexes in Kidney dialysis patients, **American Journal of Clinical Nutrition** 1996; 64: 757 – 760.

95. Van Renterghem D,Cornelis R, and Vanholder R.Behavior of 12 trace elements in serum of uremic patients on hemodiafiltration, **Journal of Trace Elements and Electrolytes in Health and Disease** 1992; 6: 169.

96. Allan C. Deacon, Sharon D. Whatley and George H. Elder. Porphyrins and disorders of Porphyrin metabolism.**Tietz textbook of clinical chemistry and molecular diagnostics** 2006, 4th edition, edited by Carl A Burtis, Edward R Ashwood, David E. Bruns, Page No.1211. Elsevier Saunders.

97. Locatelli F, D Fouque, O Heimburger, J B Cannata - Andia, W H Horl, E Ritz, Nutritional status in dialysis patients: European consensus. **Nephrology Dialysis Transplantation** 2002; 17: 563.

98. Eschbach J, Cook J D, Scribner B H, Finch C A. Iron balance in hemodialysis patients, **Annals of Internal medicine** 1977; 87: 710 – 713.

99. Carter R A, Hawkins J B and Robinson B H B, Iron metabolism in the anemia of chronic renal failure. Effects of dialysis and of parenteral iron. **British Medical Journal** 1969; 3: 206 – 210.

100. Eschbach J W, Cook J D and Finch C A, Iron absorption in chronic renal disease, **Clinical Science** 1970; 38(2): 191 **–** 196.

101. B. Brozovich, W.R. Catell, M.F.Cottrall, M.M. Gwyther, J.M. Mcmillan, J.S. Malpas, A. Salsbury and N.G.Troh. Iron metabolism in patients undergoing regular dialysis therapy. **British Medical Journal** 1971; 1: 695- 698.

PROFORMA

Stanley Medical College Hospital
Chennai.

Date :

Name :

OP/IP No:

Age :

Sex :

Occupation :

Clinical History :

On Examination

Height:

Weight:

Heart rate:

Blood Pressure:

Respiratory rate:

Cardiovascular system:

Respiratory System:

Central Nervous System:

Abdomen:

Investigation:

1. Blood Urea :
2. Serum Creatinine :
3. Serum Iron :
4. Serum Total Iron Binding Capacity :
5. Serum Ferritin :
6. Serum Transferrin Saturation :
7. Serum Transferrin :
8. Serum Copper :
9. Serum Ceruloplasmin :
10. Serum Zinc :